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INVERSION GRADIENTS AND NATURAL SELECTION IN ECOLOGICAL RACES OF DROSOPHILA FUNEBRIS

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INTRODUCTION

NATURAL populations of *Drosophila funebris*, like those of many other species of Drosophila, show variations in the gene arrangement in their chromosomes due to inversion of chromosome sections. DUBININ and TINIAKOV (1945, 1946a, 1946b, and unpublished data) have found that these chromosomal variants are by no means adaptively neutral but, on the contrary, have a profound ecological significance. Some inversions occur relatively more frequently in southern and others in northern localities, thus exhibiting climatic gradients. However, the overall frequencies of inversion heterozygotes found in a population depend on the degree of industrialization of the locality in which this population lives. Thus, the highest recorded frequencies of inversion heterozygotes have been found in the central part of the city of Moscow and in certain other industrial cities of U.S.S.R. In smaller towns, in garden-like cities like Alma Ata in Turkestan, in cities devastated by the war, and in suburban districts of large cities inversion heterozygotes are less frequent. Finally, in rural districts inversion heterozygotes are rare or even absent. Within a locality, the frequencies of inversion heterozygotes need not remain constant with time; changes from month to month connected with the seasonal succession of environments have been recorded. Experiments on hibernation of the flies have shown that some of the gene arrangements survive hibernation more frequently than others, and that the fecundity level of the survivors is higher than the average for the population before hibernation.

The above observations are strikingly paralleled by the quite independently conducted work of DOBZHANSKY (1943) and WRIGHT and DOBZHANSKY (1946) on *Drosophila pseudoobscura*. These authors have also found cyclic changes in the frequencies of inversions in populations of certain localities in California. These changes are induced by natural selection acting differently at some seasons of the year than at others. Furthermore, some of these changes have been reproduced experimentally in especially constructed population cages.

The discovery of the ecological significance of inversions found in natural populations of Drosophila opens the possibility of experimental approach to the study of evolutionary changes in free-living populations. It is possible to create in nature populations of a desired composition, and to follow the alterations which occur in such populations under the influence of natural agents. The present paper reports the results of an experiment of this kind conducted in 1945 at the Biological Station of our Institute at Kropotovo, 115 kilometers southwest of Moscow.

MATERIAL AND METHOD

Although several investigators have attempted to introduce into free-living populations certain laboratory mutants and to follow their subsequent fate, no experimental populations containing a genotypic diversity which has gone through a long process of adaptive evolution under natural conditions have ever been created in nature (WRIGHT and DOBZHANSKY (1946) however, have studied such populations in experimental cages in the laboratory).

GORDON (1935, 1939) released individuals of *Drosophila melanogaster* heterozygous for the mutant gene ebony at a certain locality in England. This species is not native to England and is introduced there accidentally by man. In 1934, GORDON placed 36,000 ebony heterozygotes into large jars. On May 15, 1934, these jars, now containing larvae and pupae as well as adults, were exposed in a garden. Flies were collected 128 days after the release at the point at which the flies had been liberated, as well as in a store and in a laboratory located within half a mile from the point of release. Eleven ebony heterozygotes were found among the 57 flies collected. Thus, the frequency of the gene ebony in the released population was 50 percent, and 128 days later it fell to between 10 and 15 percent. GORDON believes that this result is due to complete elimination of ebony homozygotes in every generation elapsed between the dates of the release and of the collecting of the flies. OLENOV and collaborators (1937) released a mixture of wild type and of the dominant mutant Bar of *Drosophila melanogaster* in the basement of a house. As could be expected, the Bar mutant was very soon crowded out by the wild type.

GERSHENSON (1941) released near Kiev some *Drosophila virilis* homozygous for the autosomal recessive veinlet (shortened wing veins). This species does not occur naturally near Kiev. The author is of the opinion that the dispersal capacity of veinlet individuals varies in proportion to the degree of the expression of this mutant. Four flies, all of them having a slight expression of veinlet, were collected 15 months after the release. GERSHENSON drew the conclusion that the veinlet character underwent an evolution in the direction of its suppression by the action of natural selection.

A different type of analysis of artificial free-living mutant populations was inaugurated by the work of the TIMOFEEFF-RESSOVSKYS (1940). These authors released known numbers of individuals of *Drosophila melanogaster* and *Drosophila funebris* marked with suitable mutant characters at the center of an experimental field consisting of traps placed checker-board fashion at ten-meter distances from each other. Fields of 63 traps (60×80 meters), or of 121 traps (100×100 meters), were used. The mutual flies barely reached the boundaries of these fields at the end of the experiments. The authors concluded that the diffusion rates of both species are so low that populations partially isolated from each other by distance may be formed on very small territories.

DOBZHANSKY and WRIGHT (1943) studied the same problem in *Drosophila pseudoobscura*. Experimental fields consisting of two lines of traps intersecting at right angles were built in localities in which the species normally occurs. The

intervals between traps were 20 meters, and the longest files of traps used were 1000 to 1400 meters long. Individuals of the mutant orange (an eye color) were released at the center of the cross-shaped experimental fields. The numbers of orange and of wild type flies that entered the traps one, two, three, etc. days after the release of the mutants were recorded. One day after the release, most orange-eyed flies were found close to the point of the release, although a few individuals had migrated for longer distances. On subsequent days the distribution curves of the orange flies became progressively more flat. An analysis of the data showed that the rate of migration of the flies is a function of temperature. Below 60°F the movements are very slow. At 70–71°F the flies traveled about 130 meters per day. The number of the mutants present on the experimental fields declined by about eight to nine percent per day. The absolute density of the wild flies is estimated to have been about 400 per 10,000 square meters in June, and 800 per 10,000 square meters in July. DOBZHANSKY and WRIGHT conclude that the rates of dispersal of *Drosophila pseudoobscura* are of a higher order of magnitude than those of *Drosophila funebris* and *Drosophila melanogaster*.

The above attempts to analyze the evolution of mutant populations have dealt with laboratory mutants. The drawback of these techniques is that the mutants involved are deleterious to the organism; they have not been subjected to evolutionary trials in nature and have not become a part of the systems of adaptive polymorphism in the species concerned. Such experiments give only one-sided models of the evolutionary changes in the populations. In our opinion, the materials chosen for studies on the interactions of ecological factors and of natural selection in free-living populations ought to come from among the genetic variants that constitute an integral part of the natural populations.

Therefore, we have made use of the inversion II-1 in *Drosophila funebris*. As shown by DUBININ and TINIAKOV (1945, 1946a, 1946b), this inversion forms a constant adaptive element in the populations of this species. It has a definite ecological optimum, and it has undergone a long evolutionary course in the species concerned. The inversion homozygotes, as well as the heterozygotes and the standard homozygotes, can be recognized readily and quite objectively in the salivary gland preparations. The strains containing this inversion were obtained from flies collected in the central part of the city of Moscow, where the II-1 inversion is very common.

EXPERIMENTS

On May 18, 1945, 10,000 *Drosophila funebris* flies known to have been homozygous for the inversion II-1 were brought to the Biological Station at Kropotovo. These parent flies were placed into three large earthenware jars with fly food medium for propagation. The jars were kept in a warm room. Early in June, the flies then present in the jars were permitted to escape outdoors. A total of about 100,000 flies (estimated by counting the puparia in the jars) were thus liberated.

Since the flies released were phenotypically identical with the wild flies of the

same species constituting the local populations, the fate of the released flies had to be followed by means of cytological examinations. The karyotype of the local Kropotovo population was first examined in 1940 by G. G. TINIAKOV. Among the 108 flies then examined, one fly was heterozygous for the inversion II-1. The frequency of heterozygotes was, therefore, 0.92 percent. A more extensive sample of the Kropotovo population was examined in 1944. Nine heterozygotes were found among 1286 flies, which amounts to 0.70 percent. These are about the normal frequencies of inversion II-1 heterozygotes in the rural areas around Moscow. The Kropotovo population is a stable rural race of *Drosophila funebris*.

In July, a sample of 114 flies was collected at the Biological Station, and an analysis of the gene arrangements in them was made. In this sample, 28 flies were inversion homozygotes, 57 flies were heterozygotes, and 29 Standard homozygotes. The gametic frequency of the II-1 inversion, consequently, is 49.5 percent, instead of the 0.35 percent present before the release of the experimental flies. It may be noted that the populations which inhabit the central part of the city of Moscow have about 50 percent frequencies of this inversion. The release of the experimental flies, therefore, has caused the appearance in the neighborhood of the Biological Station near Kropotovo of a population which has a composition typical for a large industrial city. However, this population now exists in a rural environment.

It is important to note that the inversion and Standard homozygotes and heterozygotes in the above sample have frequencies that agree very well with those expected on the basis of the Hardy equilibrium theorem, as follows:

	INVERSION HOMOZYGOTES	HETEROZYGOTES	STANDARD HOMOZYGOTES
Observed	28	57	29
Expected	28.5	57	28.5

This circumstance is very important, because it shows that the crossing of the released flies with wild ones resulted in the formation of a new random breeding population.

The Biological Station is situated close to the western margin of the village of Kropotovo. This village is bisected by a deep ravine overgrown by trees. On the other side of Kropotovo, about one kilometer distant from the Biological Station, there lies the village Besovo, separated by a cemetery and a road flanked by trees. To the west of the Biological Station, and separated by a forest with deep ravines, likewise about one kilometer distant from the Biological Station, is the village Maloe Kropotovo. These localities are inhabited by a group of interconnected micropopulations of *Drosophila funebris*. Other villages and populations of the fly lie relatively far away (5, 8, and 10 kilometers) from the above system of micropopulations. The area occupied by this system is at least 400,000 square meters (2 kilometers long and 200 meters wide).

Population samples were taken at the Biological Station in August, Septem-

ber, and October, in the near portion of Kropotovo in July, August, and September, in the more remote part of Kropotovo in July and August, in Besovo in August and September, and in Maloe Kropotovo in August and September. The results of the analyses of the chromosomes in these samples are summarized in table 1. This table shows the observed numbers of inversion

TABLE 1

Observed and expected numbers of Inversion and Standard homozygotes and heterozygotes found in different collecting localities in different months.

LOCALITY AND MONTH		STANDARD HOMOZYGOTES	HETEROZYGOTES	INVERSION HOMOZYGOTES	q	FLIES EXAMINED
Biological Station, July	Obs	29	57	28	49.5	114
	Exp	28.5	57.0	28.5		
Biological Station, August	Obs	70	52	4	23.8	126
	Exp	72.8	46.0	7.2		
Biological Station, September	Obs	144	73	1	17.2	218
	Exp	150.0	61.5	6.4		
Biological Station, October	Obs	187	43	1	9.7	231
	Exp	187.4	41.3	2.2		
Near Kropotovo, July	Obs	70	55	4	24.4	129
	Exp	73.8	47.6	7.6		
Near Kropotovo, August	Obs	80	63	7	25.7	150
	Exp	82.8	57.3	9.9		
Near Kropotovo, September	Obs	10	5	0	16.7	15
	Exp	10.8	3.9	0.3		
Far Kropotovo, July	Obs	52	16	2	14.3	70
	Exp	51.8	16.8	1.4		
Far Kropotovo, August	Obs	88	26	1	12.2	115
	Exp	89.0	24.2	1.7		
Besovo, August	Obs	37	9	0	9.8	46
	Exp	37.3	8.2	0.5		
Besovo, September	Obs	67	4	0	2.8	71
	Exp	68.2	2.8	0.05		
Maloe Kropotovo, August	Obs	12	5	0	14.7	17
	Exp	12.5	4.2	0.4		
Maloe Kropotovo, September	Obs	107	8	0	3.5	115
	Exp	109.1	5.8	0.1		

and Standard homozygotes and of heterozygotes, and the expected numbers computed according to Hardy's formula. The column marked "q" gives the gametic frequencies of the II-1 inversion in percentages. The data in table 1 indicate the diffusion rates of the inversion in the micropopulations studied, as well as the dynamics of the concentrations of this inversion at different times.

The release of the experimental flies caused the frequency of the inversion at

the Biological Station to rise from its normal value of 0.35 percent to 49.5 percent in July. The frequency in the near part of Kropotovo in July was lower than at the Biological Station—namely, 24.4 percent. In the more remote part of Kropotovo it was still lower—namley, 14.3 percent. Thus we have a clear picture of the diffusion of the inversion proceeding from the point of the release, at the Biological Station, and permeating the surrounding regions.

In August the concentration of the inversion at the Biological Station fell to 23.8 percent, and in near Kropotovo it stayed at about the same level—25.7 percent. In Far Kropotovo it was 12.2 percent, in Besovo 9.8 percent, and in Maloe Kropotovo 14.7 percent. The rate of diffusion of the inverted chromosomes through our system of micropopulations is noteworthy. In only two months after the release of the experimental flies, all the populations showed the inverted and the Standard gene arrangements to be not only present but to occur both as homozygotes and heterozygotes in relative frequencies conforming to Hardy's equilibrium. Unfortunately, we have not followed the rates of diffusion, because the high rate of dispersal which has been observed was unexpected by us. However, the rates of diffusion can be estimated indirectly. The distribution of homozygotes and heterozygotes in accordance with Hardy's equilibrium can become realized only in two generations. About 40 days are required for the development of two generations in nature. Since a random distribution has been found 60 days after the release of the flies, the released flies must have become dispersed in all populations in at most 20 days. Since the Biological Station is located in the center of the system of populations studied, the migration rate must amount to 50–100 meters per day on the average. Our evidence, consequently, contradicts that of the TIMOFEEFF-RESSOVSKYS, according to whom *Drosophila funebris* migrates about four meters per day. Nor can we agree with the inference of DOBZHANSKY and WRIGHT (1943) that *Drosophila funebris* is a much more slowly dispersing form than *Drosophila pseudoobscura*. Both species seem to migrate about equally fast.

The fact that on all months the Hardy's equilibrium proportions were realized in all localities suggests that the populations of these localities experience no appreciable migration pressure. It seems that in July, August, September, and October our system of micropopulations is broken up into separate foci without much interchange of individuals among them. This, in turn, suggests the existence of seasonal phases in the migration of the flies. Intense movements of the flies took place early in June after their liberation. It is possible that these intense movements were due to a scarcity of food and of breeding places at that time. In two or three weeks the released flies became distributed, though not uniformly, in the whole system of populations. This period of migration was then followed by a period of more sedentary existence, possibly caused by abundance of food in the environment.

Assuming that the above hypothesis of a sedentary period is correct, it follows that the changes in the incidence of the inversion which took place in subsequent months were due to the action of natural selection. Indeed, the

concentrations of the inversion fell with time (table 1). At the Biological Station, the inversion frequency was 49.5 percent in July, 23.8 percent in August, 17.2 in September, and 9.7 in October. Similar trends toward decrease of the concentration of the inversion were observed also in other localities.

Table 1 shows the observed numbers of the three possible karyotypes (inversion and Standard homozygotes and heterozygotes) in each population sample examined, as well as the expected frequencies of these karyotypes calculated with the aid of Hardy's equilibrium formula. A comparison of the observed and the expected figures indicates a systematic excess of heterozygotes and a deficiency of both classes of homozygotes. This suggests that natural selection has a differential influence on these karyotypic groups, favoring the heterozygotes at the expense of the homozygotes. Table 2 shows the

TABLE 2

Expected and observed numbers of homozygotes and heterozygotes in the combined data.

	STANDARD HOMOZYGOTES	HETERO-ZYGOTES	INVERSION HOMOZYGOTES
Observed	924	359	20
Expected	949.1	317.5	36.4
Deviation	−25.1	41.5	−16.4
Deviation in percentage of the expected number	2.8%	13%	45%

total numbers of homozygotes and heterozygotes found in all population samples except that taken in July at the Biological Station. Numbers of the homozygotes and heterozygotes expected on the basis of Hardy's equilibrium are also given. It can be seen that the deficiency of homozygotes for the Standard gene arrangement amounts to only 2.8 percent of the expected number, while that of the inversion homozygotes is 45 percent. In other words, the negative selection directed against the inversion homozygotes is sixteen times stronger than that against the Standard homozygotes.

The heterozygotes are favored by the natural selection at work in the populations studied. The excess of the heterozygotes amounts to 13 percent of their expected number. It is evident that we are dealing here with a case of an intrapopulational heterosis. The increased survival of heterozygotes retains the newly introduced inversion in the population. The selection directed against the inversion homozygotes, however, is so intense that all the populations undergo changes toward decreases of the frequencies of the inversion.

DISCUSSION

If individuals of *Drosophila funebris* homozygous and heterozygous for the inversion II-1 and for the corresponding Standard gene arrangement are induced to hibernate, inversion homozygotes and heterozygotes are discriminated against by natural selection to almost the same degree (DUBININ and TINIAKOV 1946a). Here, then, the genetic contents of the inversion act as a dominant to those of the Standard chromosomes. But the action of selection is

quite different in summer, particularly during the period when the populations of the species expand in numbers. For at this time the Inversion/Standard heterozygotes possess the highest adaptive value, the inversion homozygotes the lowest, and the Standard homozygotes an intermediate one. This difference in the action of natural selection during hibernation on one hand and during the time of the expansion of the populations in summer on the other shows how complex may be the influence of natural selection on the genetic structure of populations in nature. It may be noted here that while in the city populations the frequency of the inversion heterozygotes waxes from month to month during the summer, this is not the case in rural populations. In the latter the concentration of the inversion is lower than in the city, and it does not change appreciably during the summer. A somewhat analogous situation has been discovered by DOBZHANSKY (1943) and WRIGHT and DOBZHANSKY (1946) in *Drosophila pseudoobscura;* here certain inversion heterozygotes also possess an adaptive value much higher than the corresponding homozygotes during a part of the annual seasonal cycle (spring and summer), while during other seasons (autumn and winter) they seem to be equivalent. Furthermore, the least favored homozygote during the spring season becomes the relatively more favored one during the summer.

The data presented in this paper establish, independently from the similar findings of WRIGHT and DOBZHANSKY (1946), the fact of intrapopulational heterosis involving an inferiority of inversion homozygotes to inversion heterozygotes. This confirms the theoretical conclusions reached by STURTEVANT and MATHER (1938), MALINOVSKY (1940), WRIGHT and DOBZHANSKY (1946), and others. S. J. BESSMERTNAYA (unpublished), working in our laboratory, obtained in 1939–1940 experimental results indicating that in *Drosophila melanogaster* there is a preferential survival of heterozygotes for the inversions CIIL–CIIR. In these experiments she used artificial populations kept in population cages of the type proposed by L'HERITIER and TEISSIER. Experiments of this type have been more recently developed by WRIGHT and DOBZHANSKY (1946).

The data on the intrapopulational heterosis prove that the persistence of low concentrations of inversions in rural populations of *Drosophila funebris* is caused by natural selection favoring the respective inversion heterozygotes, with selection against the homozygotes inhibiting the growth of the concentrations of these inversions. Although it is possible that the interrelations between the environment and the inversions are more complex than indicated above, the importance of heterosis is evident.

SUMMARY

In early June, 1945, a total of about 100,000 *Drosophila funebris* flies homozygous for a certain inversion were liberated at the Biological Station near Kropotovo, 115 kilometers southwest of Moscow. In July, the descendants of the released flies were present in the populations of localities about one kilometer distant from the point of release. The relative frequencies of the

inversion homozygotes and heterozygotes was found to be approximately that expected on the basis of the Hardy's equilibrium formula, with the heterozygotes being somewhat more frequent. It is estimated that the rate of diffusion of *Drosophila funebris* during the early part of the summer amounts to about 50–100 meters per day on the average.

Natural selection favors the inversion heterozygotes more than it does the Standard homozygotes, and the latter more than the inversion homozygotes. We are dealing here with a case of intrapopulational heterosis. This phenomenon is very important in the dynamics of natural populations. The action of natural selection, however is, far more complex than the data on the intrapopulational heterosis would by themselves suggest. Thus, during hibernation both inversion homozygotes and heterozygotes are discriminated against in favor of the Standard homozygotes. The action of natural selection is also different in rural and in urban populations.

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CHROMOSOMAL VARIATION IN DROSOPHILA NEBULOSA*

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INTRODUCTION

INDIVIDUAL and racial variations in the gene arrangement of chromosomes occur in many animal and plant species. Among the sexually reproducing and cross fertilizing organisms most of the variations are caused by inversion of sections of chromosomes. Other classes of chromosomal aberrations, such as translocations, duplications, and deficiencies, occur much less frequently than the inversions, doubtless because the former are eliminated by natural selection owing to the unfavorable effects on the viability and fertility which most of them produce. The technique of the detection of inversions varies from organism to organism. Where, as in many Diptera, the giant chromosomes in the salivary gland cells are available for study, the inversions can be identified by observing the loop-like configurations formed in heterozygotes, or by examining the pattern of the stainable discs in inversions homozygotes. Inversion heterozygotes can also be identified at meiosis through the formation of the chromatid bridges and acentric fragments in cells in which crossing over takes place within the inverted sections. The genetic method of the detection of inversion through the apparent suppression of crossing over in inversions heterozygotes is applicable only to species in which groups of linked genes are known.

Flies belonging to the genus Drosophila have been more thoroughly studied with regard to inversions than any other group of organisms. These studies disclosed the remarkable fact that the characteristics of the chromosomal variability vary from species to species. Natural populations of some species appear to be uniform with respect to the gene arrangement in all chromosomes. Conversely, in other species the gene arrangement is so variable that a majority of individuals encountered in natural habitats are inversions heterozygotes. In some species the inversions occur mainly or exclusively in a single chromosome, while in others several chromosomes are subject to inversions. Observations on the variation in the gene arrangement in the chromosomes of *Drosophila nebulosa* Sturtevant are reported in the present article. The remarkable feature of this species is that eight out of the nine inversions so far found in natural populations are concentrated in a single chromosome, which happens to be the shortest in the chromosome set.

MATERIAL AND METHODS

D. nebulosa Sturtevant is a species which is rather common in tropical and partly subtropical America. Its known distribution area extends from Florida

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[1] Research Fellow of THE ROCKEFELLER FOUNDATION. Work reported at the first Reunião Conjunta dos Sociedades Brasileiros de Biologia.

and central Texas southward to the state of São Paulo in Brazil. Strains from the following localities were used in the present investigation: Del Rio, Texas (collected by PROF. J. T. PATTERSON); Prata (collected by DR. ROSINA DE BARROS); São Jose dos Campos (collected by MISS MERCEDES RACHID); Ilhabella and Santos (collected by MISS RUTH MORRETES and MR. ANTONIO BRITO DA CUNHA); Bertioga and Iporanga (collected by MR. HENRIQUE DE OLIVEIRA and the writer) in the state of São Paulo; and Belem (collected by PROF. TH. DOBZHANSKY and the writer) in the state of Pará; all but the first locality are in Brazil.

The strain from Del Rio was chosen as the Standard, because in this strain only two easily identifiable variations in the gene arrangement have been found. Flies from all others strains were crossed to Del Rio in small mass cultures. The hybrid larvae were grown under optimal food and temperature (from 19° to 24°C) conditions, and their salivary glands were stained in aceto-orcein. All the preparations were examined when fresh. A supplementary study was made of chromosomes within each strain, and also in the hybrids between the Bertioga strain and most other strains.

MITOTIC AND SALIVARY CHROMOSOMES

D. nebulosa has in its somatic cells two pairs of V-shaped and one pair of rod-shaped chromosomes. The X and the Y form one of the V-shaped pairs, the other V and the rod-shaped chromosomes being autosomes (see Plate I).

The salivary gland chromosomes of *D. nebulosa* show five euchromatic strands attached to a large and compact chromocenter. It is possible to individualize the chromosomes by breaking the chromocenter through sufficient pressure on the cover slip (BAUER 1936). This procedure shows that almost the entire chromocenter is formed by the second chromosome (the V-shaped autosome), while the X and the rod-shaped autosome form only a very small part of it. Using two translocations involving the second and the Y chromosomes, PAVAN (1946) showed that the heterochromatic portions of the second chromosome on the one hand, and of the X and the third (rod-shaped) chromosome on the other, are qualitatively distinct. In the mitotic prophase of the giant nerve cells of the larval brain the X and the second chromosomes show approximately equally long and similarly staining heteropycnotic sections. The Y chromosome is entirely heteropycnotic. Yet, the heteropycnotic sections of the X and Y chromosomes are reduced in the salivary gland cells to very small blocks of the chromocentric material while the heteropycnotic part of the second chromosome gives rise to a large foamy mass of the chromocenter.

The relative lengths of the euchromatic parts of the salivary gland chromosomes were measured, with the aid of an "K and E map measure," on camera lucida drawings of at least 20 specimens of each chromosome. The following average values have been obtained (expressed in percentages of the combined lengths of the euchromatic parts of all chromosomes). The X chromosome has 35.8 percent, the left limb (XL) being 17.1 percent and the right limb (XR) 18.7 percent. The second chromosome has 39.1 percent, the left limb IIL

amounting to 19.1 percent and the right limb (IIR) to 20.0 percent. The third chromosome has 25.1 percent. The relative lengths of the chromosomes shown in Plate I are not typical.

Plate I shows composite maps of the five euchromatic strands. Because of the difficulty of obtaining an entire set of very favorably extended chromosomes in a single preparation, the maps were made by combining parts of the chromosomes drawn from different cells. After the chromosome maps were completed, they were compared with chromosomes in cells other than those used for making the drawings. The distal third of the left arm of the second chromosome is very difficult to study, because it contains several sections which exhibit nonspecific attraction and nonhomologous pairing causing this part of the chromosome to be convoluted and compressed in most cells.

For convenience of description, the salivary chromosome complement, following the example of BRIDGES and others, was divided in 100 arbitrary sections. As shown in Plate I, the X chromosome has 35 sections (from 1 to 35), the second chromosome 40 sections (from 36 to 75), and the third chromosome 25 sections (from 76 to 100). Since variations in the gene arrangement have been found chiefly in the third chromosome, the limits of the sections in this chromosome only are shown in Plate I.

X chromosome

As mentioned above, the X chromosome contributes only a very small part of the chromocenter in the salivary gland cells. It often shows its arms separated from each other, broken free from the chromocenter, and sometimes associated with the third chromosome. The arrow below the map of the XL (Plate I) indicates a point at which this chromosome is frequently broken in preparations. Beside its characteristic base and tip, this chromosome can be identified by the negligible quantity of heterochromatic material near the centromere and by the facility with which it breaks free from the chromocenter.

Chromosome II

This is the chromosome that forms almost the entire chromocenter, and its arms are always associated with this structure. Each arm contributes an approximately equal portion of heterochromatin to the formation of the chromocenter; this can be seen when, by pressure on the cover slip, the chromocenter breaks and each arm retains the part formed by it. As mentioned above, the left arm has several regions which exhibit nonspecific pairing. It is not difficult to find a cell in which the other four euchromatic strands are well extended but the IIL is convoluted. The region between the two arrows in the distal part of IIL appears to be euchromatic in some cells and heterochromatic in other cells; such variations have also been found in some regions of the X chromosome of *Drosophila melanogaster* (PROKOFIEVA-BELGOVSKAYA 1945) and in chromosomes of *Drosophila pallidipennis* (DOBZHANSKY 1944a). This condition makes the drawing of a map of this region difficult. The three arrows pointing at parts of the IIL indicate areas of the chromosome that have a very

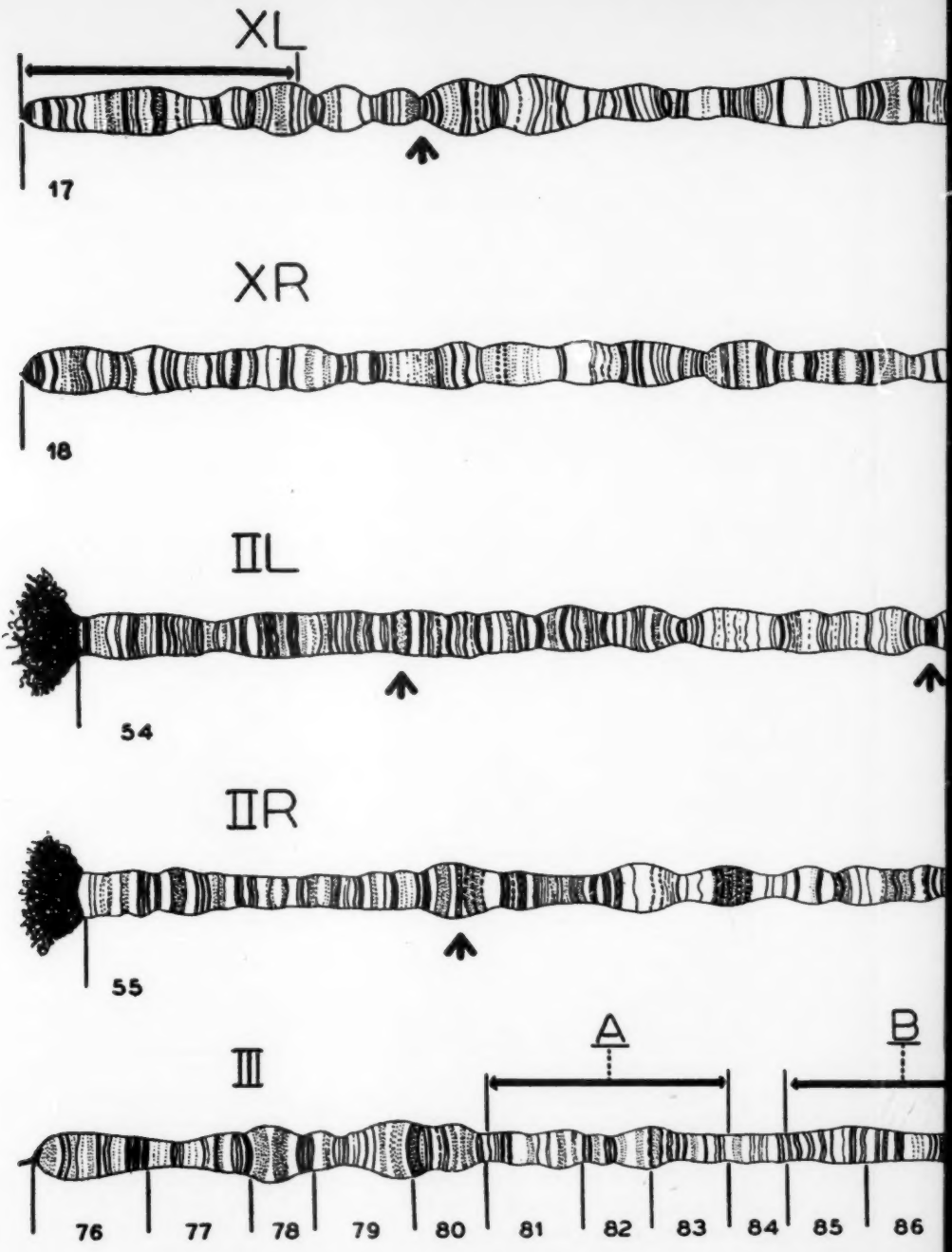
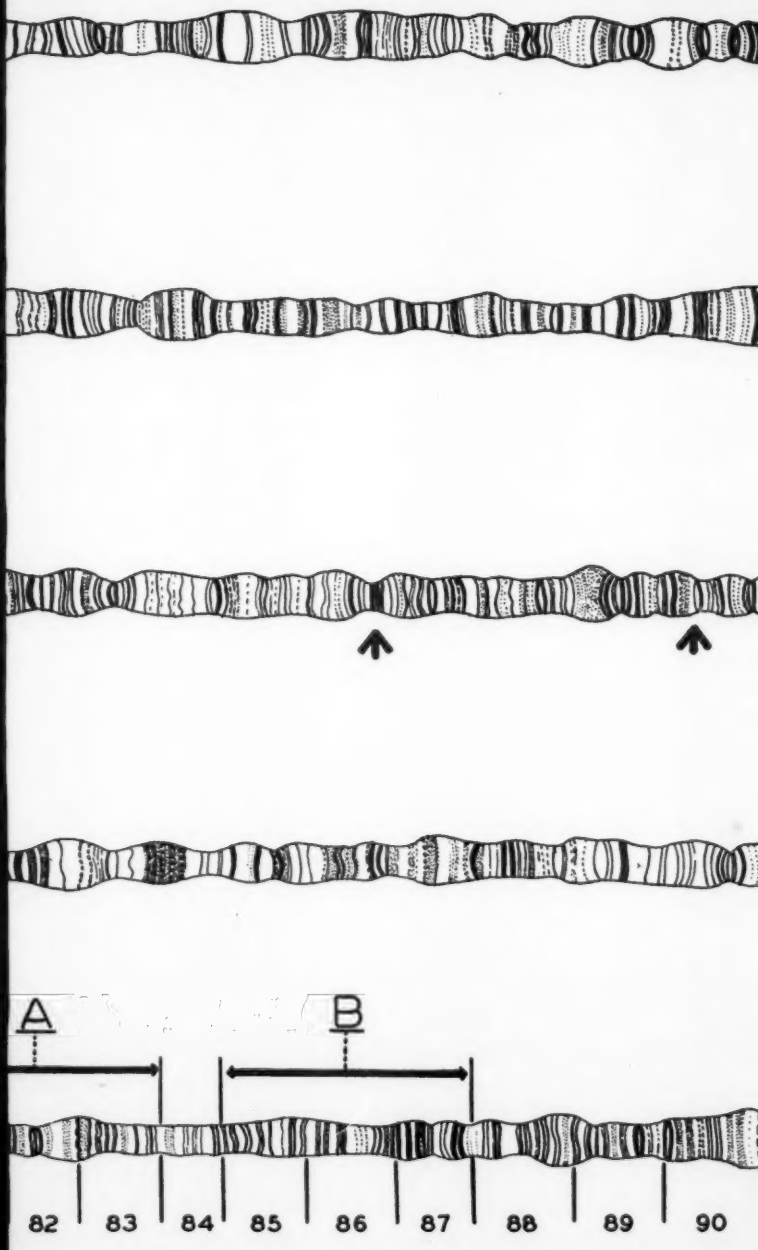
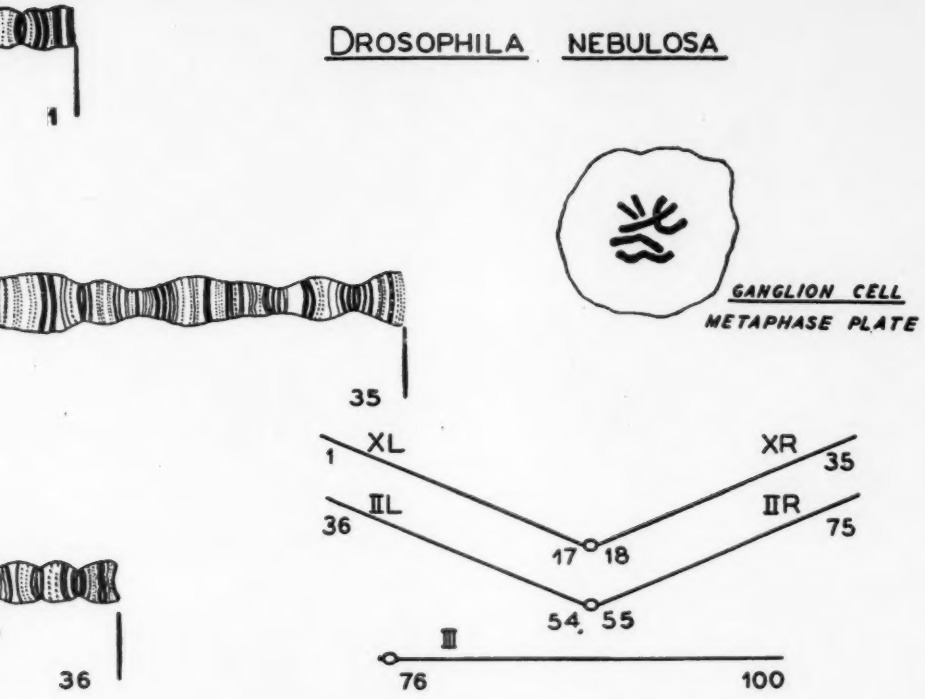


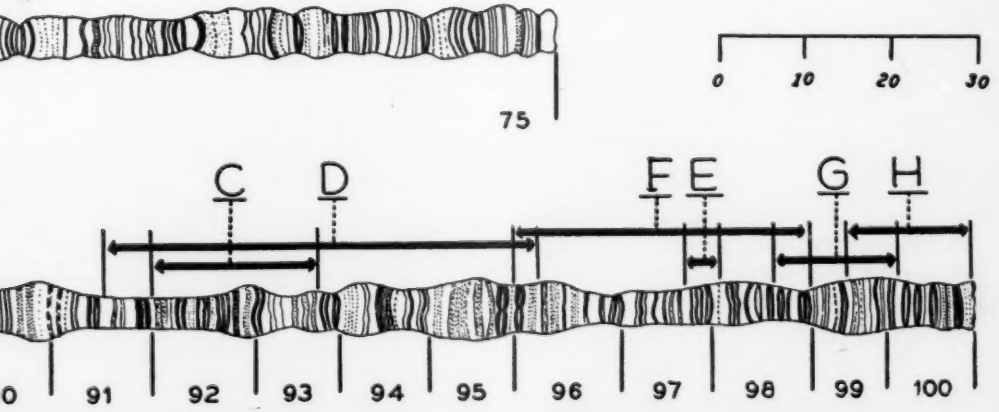
PLATE I.—Composite Standard maps of the salivary gland chromosomes of *Drosophila nebulosa*. The s
is shown for comparison. The limits of the inversions



nd chromosomes of *Drosophila nebulosa*. The scale represents 30 micra. A figure of metaphase
wn for comparison. The limits of the inversions found in the XL and in the third chromosom

DROSOPHILA NEBULOSA





phase plate chromosomes in a larval nerve cell, drawn at the same scale as the salivary gland chromosomes, mosomes are shown by black lines with double arrows.

characteristic appearance and serve as landmarks for recognition of this chromosome. The most distal arrow (close to section 36) indicates a point of easy breakage; the region of the chromosome between the arrow and the free end is always very distinct. The right arm (IIR) is often well extended and easily recognizable. The arrow points at a swollen region very characteristic of this chromosome.

Chromosome III

Like the X chromosome, this chromosome contributes little chromocentral material and for this reason often breaks loose from the chromocenter. In some cells this chromosome seems to show a connection with the base of one of the arms of the X chromosome. With the exception of section 90, and of parts of sections 89 and 91, which form a region of repeats, this chromosome is very clear in many cells. Section 78 is swollen to different degrees in different cells; this section, together with the adjoining base of the chromosome, constitutes an easily identifiable landmark.

DESCRIPTION OF THE INVERSIONS

The gene arrangement in both limbs of the second chromosome and in the right limb of the X chromosome proved to be uniform in all strains examined.

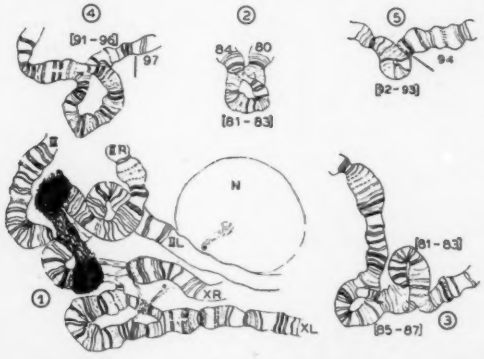


FIGURE 1.—The bases of the five chromosome limbs showing the attachment to the chromocenter. The left limb of the X chromosome (XL) has an inversion with one break in the heterochromatin. N=nucleolus. Figure 2.—Inversion A. Figure 3.—Inversions A and B. Figure 4.—Inversion D. Figure 5.—Inversions C in the third chromosome. The numbers indicate the sections of the Standard chromosome map shown in Plate I.

Contrasting with this constancy, the third chromosome is very variable.

The left limb of the X chromosome (XL) is uniform in all strains except Del Rio. In approximately five larvae among hundreds examined from the strain just named, there was found an inversion close to the base of XL. This inversion is shown in Figure 1. It includes approximately the basal fifth of the length of the chromosome arm. The remarkable feature of this inversion, which

is unique among the inversions so far encountered in natural population of any species of Drosophila, is that one of its breaks lies in the euchromatin and the other in the chromocenter. Chromosomal aberrations induced by X-ray treatment in Drosophila frequently involve breaks in the heterochromatic regions which contain the centromeres (BAUER, DEMEREC, and KAUFMANN 1938; HELFER 1941). Nevertheless, such aberrations have not been found in natural populations, although they must have occurred in the evolution of *Drosophila miranda* (MACKNIGHT 1939). If they arise in nature as well as in X-ray experiments, they must be eliminated by natural selection. Why such an elimination takes place is not completely clear. According to SCHULTZ (1941) and others, transfers of genes normally located far from heterochromatic sections to the proximity of such sections, or vice versa, are frequently accompanied by position effects. Position effects, like mutations, may be deleterious. It may be noted in this connection that our XL inversion is found in a single strain, and only in a minority of the flies in that strain. It is possible but not probable that this inversion arose spontaneously in the laboratory and consequently was not present in the wild progenitor of the Del Rio strain. The strain was collected by PROFESSOR J. T. PATTERSON in 1942 and kept in laboratory cultures since that time.

In the third chromosome, 17 different gene arrangements have been found. These gene arrangements are obtained as a result of combination of eight different inverted sections. In the Del Rio strain, which has been selected as the standard, three types of larvae occur having different conditions in the third chromosome. One type shows an inversion including the distal half of the section 99 and the section 100 (fig. 6, 7, 10). This inversion, referred to as inversion H, appears to be terminal, although it is of course impossible to be sure that one of the breaks is not really subterminal. The two other types of larvae are structurally homozygous for one or the other of the two gene arrangements involved in the inversion just described. The gene arrangement shown in Plate I has been arbitrarily chosen as the Standard; the other gene arrangement is shown in homozygous condition in figure 11, and heterozygotes with the Standard in figures 6, 7, and 10.

In crosses between the standard Del Rio strain and all the Brazilian strains, some of the larvae showed an inversion denoted as inversion A, which includes sections 81 to 83 in the sub-basal portion of the third chromosome. This inversion is shown in figures 2 and 3.

Another inversion, denoted as B, is shown in figure 3. It includes sections 85 to 87, and therefore lies distally from the inversion A, without overlapping the latter. Some of the hybrids between Iporanga and Del Rio, and between Bertioga and Del Rio strains, showed both inversions A and B simultaneously. No case of inversion B without A has been found. However, chromosomes exist with the inversion A without B.

Inversion C is shown in figure 5. It lies at about the distal two thirds of the length of the chromosome and covers the entire section 92 and the distal three-fourths of the section 93. It has been found in hybrids between the standard Del Rio strain and all the strains from southern Brazil.

Inversion D is much longer than C and includes the latter. Inversion D extends from the middle part of section 91 to the proximal fifth of 96 (fig. 4). This inversion appeared in our material only in some of the hybrids between the strain from Belem and the standard strain. The individuals which show inversion D never have inversion C described above.

Inversion E is very short and includes only the distal quarter of section 97 and the proximal tenth of 98 (fig. 8). It has been found only in the few hybrids between the strain from Ilhabella and the standard strain.

Inversion F has been found in some hybrids between the strain from Santos and the standard strain. It covers sections 96 to 98 (fig. 6), and therefore includes inversion E.

Inversion G lies close to the distal end of the chromosome. It includes the

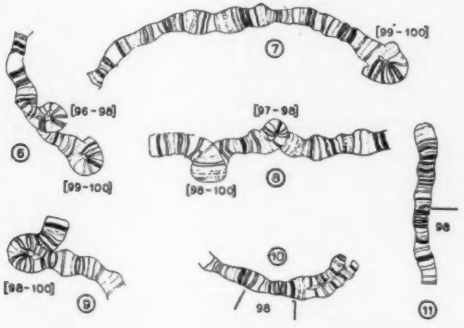


FIGURE 6.—Inversions F and H. Figure 7.—Inversions E and G. Figure 9.—Inversion G. Figure 10.—An inversion heterozygote H/h showing lack of pairing in the inverted region. Figure 11.—The distal end of the third chromosome homozygous for the inversion H. The numbers indicate the sections of the Standard chromosome map shown in Plate I.

distal third of section 98 to the proximal tenth of section 100 (fig. 8, 9). It has been found in some of the hybrids between Prata, São Jose dos Campos, Ilhabella, and Santos strains.

The above described inversion H, which is possibly terminal, has been found in the standard Del Rio strain as well as in hybrids between it and other strains from Belem, Bertioga and Ilhabella.

GEOGRAPHIC DISTRIBUTION AND THE RELATIONSHIPS OF THE INVERSIONS

The relationships between different inversions occurring in the same chromosome have been analyzed by DOBZHANSKY and STURTEVANT (1938), and DOBZHANSKY (1944). The chromosome sections involved in different inversions may be included within each other (included inversions), may overlap the limits of each other (overlapping inversions), or may lie wholly outside each other (independent inversions). Included, overlapping, and independent inversions have been found in the third chromosome of *D. nebulosa*. Overlapping

inversions are especially interesting, because their phylogenetic relationships may be inferred with a high degree of certainty. For example, assume that three gene arrangements are found in nature having the gene sequence abcdefgh, aedcbfgh, and aedgfbch respectively. The first of these gene arrangements can be related to the third only through the second. If the second gene arrangement is unknown its existence may be postulated theoretically.

Among the above described inversions in the third chromosome of *D. nebulosa*, inversions G and H are overlapping (see Plate I). With the aid of the theory of overlapping inversions their phylogenetic relationships may be established as

G↔Standard↔H

The standard gene arrangement is an intermediate step between G and H. For the sake of convenience, we may designate the Standard gene arrangement modified by each inversion by its corresponding lower case letter. Thus the Standard gene arrangement in the free end of the third chromosome becomes h (Plate I), while the gene arrangement arising by means of the inversion may be symbolized as H (fig. 11). The configuration found in the inversion heterozygotes (fig. 6, 7, 10) may thus be symbolized as H/h. Similarly, the Standard gene arrangement in section 98, 99, and part of section 100 (Plate I) is symbolyzed by g, and the gene arrangement modified by the inversion as G; G/g heterozygotes are shown in figures 8 and 9. In terms of this symbolism we may, therefore, have the relationships of the three gene arrangements written thus:

Gh↔gh↔gH

Inversion F is overlapping G, but is independent of H. The phylogenetic relationships are, therefore, as follows:

fGh↔fgh↔fgH
↕
Fgh

The Standard is the only possible link between the other three gene arrangement and very likely also the phylogenetically ancestral arrangement. Here it may be noted that since the inversions F and H do not overlap each other, a gene arrangement may theoretically arise by crossing over between Fgh and fgH arrangements, without the Standard fgh being the intermediate link. An FgH arrangement, however, has not been found in nature; in view of the proximity of the distal break in F to the proximal one in H, it is doubtful if crossing over between them could take place.

Inversion E and F (fig. 8, 6) are related as included inversions. Theoretically, these gene arrangements may arise from each other in the following two ways:

Ef↔ef↔eF, or

EF by double crossingover with ef giving rise to Ef and eF.

Since the gene arrangement EF has not been found, the first hypothesis is more probably correct than the second. Taking into account the gene arrangement in the distal portion of the chromosome, we may write:

EfGh↔efGh↔efgh↔efgH
↕
eFgh

Only the gene arrangements EfGh, efGh and efgh are found, while the theoretically possible arrangement Efgh is unknown. This makes it probable, although not absolutely certain, that EfGh arose as shown in the above diagram, rather than by crossing over between Efgh and efGh.

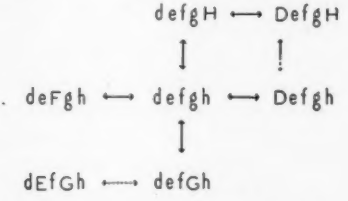
Inversion C is included in D (fig. 5, 4). Their phylogenetic relationships are probably:

Cd↔cd↔cD,

because the gene arrangement CD from which Cd and cD could arise by double crossing over with cd is unknown. Inversion D overlaps F (Plate I), hence the relationships are certainly as follows:

Df↔df↔dF

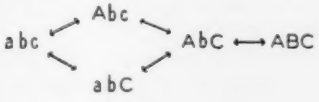
However, D does not overlap E, G, or H, and C does not overlap any of the inversions lying in the distal part of the chromosome. Their phylogenetic relationships, therefore, are ambiguous. Indeed, gene arrangement Cdefgh, CdefGh, CdefgH, CdeFgh, CdefGh, CdEfGh, cDefgh, cDefgH, cdefGh, cdefgH, and cdefgh have been recorded (see table 1). It is obvious that crossing over between C and H, and between C and G, has taken place. Inversion C cannot, therefore, be included in the phylogenetic scheme. The relationships between D, E, F, G and H are as shown below:



Inversion A and B lying in the proximal half of the chromosome are independent of each other and of all the distal inversions (fig. 2 and 3). Gene arrangements ab, Ab and AB, but not aB, have been found. This makes it probable, but by no means certain, that these gene arrangements are related thus:

ab↔Ab↔AB,

but the possibility of AB arising by crossing over between Ab and aB is not excluded. That crossing over between A and C has taken place in nature follows from the fact that all four possible gene arrangements, ac, Ac, aC and AC, have been found. The relationship between A, B and C may, therefore, be represented as follows:



There are two alternative paths between abc and ABC, namely through Abc or through abC.

All the 17 gene arrangements observed in the third chromosome of *D. nebulosa* are tabulated in table I, together with the names of the localities in which each of them was found. The Standard chromosome is represented as abcdefgh and the inverted parts are shown in capital letters (cf. Plate I).

TABLE I

1)	a	b	c	d	e	f	g	h	—(Standard) Del Rio, and Belem
2)	a	b	c	d	e	f	g	H	—Del Rio and Belem
3)	a	b	c	d	e	f	G	h	—Prata
4)	a	b	c	D	e	f	g	H	—Belem
5)	a	b	C	d	e	f	g	H	—Ilhabella
6)	a	b	c	D	e	f	g	h	—Belem
7)	a	b	C	d	e	f	g	h	—Ilhabella
8)	a	b	C	d	E	f	G	h	—Ilhabella
9)	A	B	C	d	e	f	g	H	—Iporanga
10)	A	b	C	d	e	f	g	H	—Ilhabella and Bertioga
11)	A	b	C	d	e	f	G	h	—Prata, São Jose dos Campos and Santos
12)	A	b	C	d	e	F	g	h	—Santos
13)	A	B	C	d	e	f	g	h	—Bertioga
14)	A	b	C	d	e	f	g	h	—Bertioga, Ilhabella, São Jose dos Campos, Prata and Iporanga
15)	A	b	c	D	e	f	g	h	—Belem
16)	A	b	c	d	e	f	g	H	—Iporanga and Belem
17)	a	b	C	d	e	f	G	h	—Ilhabella and Prata

It is remarkable that some of the inversions show a geographic distribution coterminous with the known distribution of the species. Thus, inversion H, as well as its alternative Standard arrangement h, are found from Texas (Del Rio) to southern Brazil inclusive. Inversion A occurred in Belem as well as in southern Brazil. On the other hand, inversion D has been found only in Belem (equatorial Brazil), and B, C, E, F, and G only in southern Brazil. However, since only eight strains of *D. nebulosa* have been studied, these apparently endemic gene arrangements may have a much wider distribution than appears from our data.

DISCUSSION

Natural populations of some species of Drosophila are uniform or nearly so with respect to the gene arrangement in their chromosomes. PATTERSON, STONE, and GRIFFEN (1940) and WARTERS (1944) found no chromosomal variations among 3598 examined chromosomes of *D. virilis* coming from strains collected in localities as remote as China, Japan, and various parts of the United States. On the other hand, in *D. americana*, *D. montana*, and *D. texana*, species closely related to *D. virilis*, the same authors have found a considerable variety of gene arrangements in a much smaller number of strains examined. At first sight this may seem to be correlated with the fact that *D. virilis* is a species associated with man, while the others are "wild" species. Individuals of *D. virilis* are doubtless transported unintentionally with fruits and vegetables, so that its populations, particularly in the Western Hemi-

sphere where this species is not native, may be descended from very few progenitors. However, other species of Drosophila, which in a major part of their present distribution area are associated with man at least as intimately as *D. virilis*, show variations in the gene arrangement. *D. melanogaster* (STURTEVANT 1931; DUBININ, SOCOLOV, and TINIAKOV 1937; WARTERS 1944) and *D. ananassae* (KAUFMANN 1936; KIKKAWA 1938; DOBZHANSKY and DREYFUS 1943) are species native presumably in southeastern Asia but now distributed much more widely: the former is nearly cosmopolitan and the latter pantropical. The populations of these species show many variations in the gene arrangement; a consequence of the association with man, however, may be seen in that populations which inhabit most remote parts of the world frequently contain the same inversions. WHARTON (1942) found no inversions in several strains of *D. repleta*, and WARTERS (1944) found only a single inversion in *D. hydei* and none in *D. meridiana* and *D. bifurca* belonging to the *repleta* species group.

In the species in which inversions do occur, they may be concentrated in a single chromosome or may be distributed more or less uniformly in the entire chromosome set. The most striking instance of the concentration of inversions is *D. pseudoobscura*. DOBZHANSKY (1944b) found 15 gene arrangements in the third chromosome of this species, three in the second chromosome, two in the fourth, and two in the X chromosome. Natural populations almost everywhere contain a high proportion of inversion heterozygotes in the third chromosome, while the second and the fourth chromosomes are with rare exceptions constant; the inversion in the X chromosome is associated with the "sex-ratio" phenomenon. The related species *D. persimilis* shows seven gene arrangements in the third chromosome, three in the second, one in the fourth and two in the X chromosome. Again, inversion heterozygotes in the third chromosome are widely distributed, while the second chromosome varies only in a small part of the geographic area of the species, and the X chromosome inversion is associated with the "sex-ratio" condition. In another member of the *obscura* group—namely, *D. miranda*—the third chromosome (X^2) is again most variable (KOLLER 1939). *D. nebulosa* also shows a striking concentration of the variations in the gene arrangements in a single chromosome. As indicated above, 17 gene arrangements have been found in the third chromosome, while other chromosomes are constant (with the exception of a single inversion in the X chromosome, which may have arisen in the laboratory). It may be noted here that *D. nebulosa* is a member of the *willistoni* group of species which is not very closely related to the *obscura* group. Whether or not the third chromosome of *D. nebulosa* is even partially homologous to the third chromosome of *D. pseudoobscura* and its relatives is not known.

Examples of species which frequently show inversion heterozygotes in several chromosomes, apart from those mentioned above are: *D. melanogaster*, *D. ananassae*, *D. americana*, *D. texana*, and *D. montana*, also *D. azteca* (DOBZHANSKY and SOCOLOV 1939); *D. algonquin* (MILLER 1939) and *D. robusta* (CARSON and STALKER 1946). *D. willistoni*, which is a relative of *D. nebulosa*, shows inversions in more than a single chromosome (unpublished data).

PHILIP, RENDEL, SPARWAY and HALDANE (1944) found inversions in five chromosome limbs of *D. subobscura*, which is a close relative of *D. pseudoobscura* and *D. persimilis*.

The reason why variations in the gene arrangement may in some species be concentrated in a single chromosome and in related species be widespread is not known. BAUER, DEMEREC and KAUFMANN (1938) in *D. melanogaster* and HELFER (1941) in *D. pseudoobscura* found that chromosome breakages induced by X-rays are distributed more or less uniformily in proportion to the chromosome length. If spontaneous breaks which lead to the formation of inversions in natural populations are likewise uniform, then it follows that natural selection favors the spread of inversions in some chromosomes but not in others. Natural selection also discriminates against inversions having one break in the heterochromatin.

The evolutionary role of inversions found in natural populations of species of Drosophila apparently depends upon the heterosis observed in at least some inversion heterozygotes. DOBZHANSKY (1943) found that the frequencies of inversions in natural populations of *D. pseudoobscura* from certain localities in California undergo cyclic changes from month to month connected with the seasonal cycle. The agent that causes these changes is natural selection. WRIGHT and DOBZHANSKY (1946) have shown experimentally that, in some environments, inversion heterozygotes in *D. pseudoobscura* have adaptive values much higher than the corresponding homozygotes. The fact that every strain of *D. nebulosa* studied in this paper proved to contain inversion heterozygotes suggests that the situation in this species may be similar to that found in *D. pseudoobscura*. This hypothesis must be tested by future work.

SUMMARY

The gene arrangement in the chromosomes of eight strains of *D. nebulosa* coming from Texas, from equatorial Brazil, and from southern Brazil has been examined. The species has three pairs of chromosomes: a V-shaped X chromosome, a V-shaped and a rod-like autosome. In the salivary gland cells five long euchromatic strands are found. The gene arrangement in the V-shaped autosome has been found to be constant. That in the X chromosome is constant, except for a single inversion found in some individuals of one strain from Texas. One of the points of breakage in this inversion lies in the chromocentral heterochromatin. The gene arrangement in the rod-like autosome (the third chromosome) is very variable. Seventeen gene arrangements in this chromosome have been identified; they arise through recombination of eight separate inversions. Some groups of inversions are overlapping; this fact permits construction of schemes showing the probable descent relationships of the different gene arrangements. Among the inversions, some are geographically widely distributed while others have been found in only a single strain.

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GENETICS OF SEXUAL ISOLATION BETWEEN *DROSOPHILA PSEUDOOBSCURA* AND *DROSOPHILA PERSIMILIS*

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INTRODUCTION

NOTWITHSTANDING their morphological similarity, *Drosophila pseudoobscura* and *Drosophila persimilis* are distinct species. No hybrids between them have been recorded in natural populations, although the two species frequently occur together in the same habitats. Although in laboratory experiments species hybrids may be obtained without much difficulty, intraspecific matings succeed more easily than interspecific ones. The principal if not the only cause of this is the pronounced sexual isolation between the species. If females of the two species are placed together with males of one of them, a greater proportion of conspecific than of alien females are inseminated (DOBZHANSKY and EPLING 1944).

Although the degree of sexual isolation between *D. pseudoobscura* and *D. persimilis* depends upon the environment in which they live (MAYR and DOBZHANSKY 1945), there can be no doubt that the principal cause of the isolation is genetic. The genetic basis of sexual isolation has never been studied. The experiments reported in the present article represent an attempt to explore this little known field. Since female hybrids between *D. persimilis* and *D. pseudoobscura* are fertile when backcrossed to either parental species, it is, theoretically, possible to obtain individuals with any combination of chromosomes of the parents. The sexual preferences of such individuals of mixed parentage may, then, permit inferences regarding the genetic mechanisms underlying the isolation between the species. It may be noted that differences in sexual preferences sometimes occur between mutants and the wild type of the same species (SPETT 1931; NIKORO, GUSSEV, PAVLOV, and GRIASNOV 1935; RENDEL 1945), as well as between geographic strains of a single species (DOBZHANSKY and KOLLER 1938; STALKER 1942; DOBZHANSKY and MAYR 1944; DOBZHANSKY and STREISINGER 1944). However, these differences in sexual preferences seldom assume the character of a true sexual isolation—that is, of a regular preference for endogamic, as opposed to exogamic, mating. This of couse, is what would be expected on theoretical grounds if, as is usually supposed, species arise from geographic races by a process of gradual divergence. The genetic elements from which an isolation may be built are available within a species, but these elements are combined to form a functional isolating mechanism only during the process of speciation—that is, during the transformation of the diverging races into distinct species.

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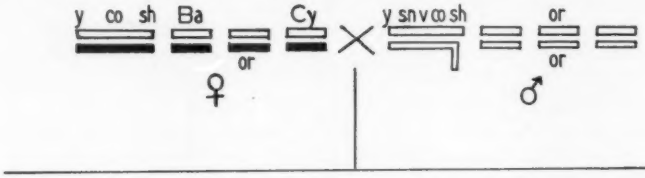
MATERIALS AND METHODS

The following mutant strains of *D. pseudoobscura* were used: (1) yellow, singed, vermilion, compressed, short (*y, sn, v, co, sh*), (2) orange (*or*), (3) orange purple (*or pr*), (4) white (*w*), (5) aristapedia (*ast*), and (6) Bare Curly (*Ba Cy*). Mutants affecting different parts of the body are included in this list: a body color mutant yellow; eye color mutants vermilion, orange, purple, and white; bristle mutants singed and Bare; wing mutants short and Curly; and an antenna mutant aristapedia. With the exception of Bare and Curly, which are dominant and lethal when homozygous, all other mutants are recessive and viable in homozygous condition. The wild type flies used were obtained from a Piñon Flats (California) strain.

In the experiments on different classes of backcross hybrids it was necessary to obtain strains of the two species, *D. pseudoobscura* and *D. persimilis*, which carried appropriate mutant markers in each chromosome except the small fifth which is not under genetic control. *D. pseudoobscura* females carrying sex-linked recessives yellow (*y*), singed (*sn*), vermilion (*v*) compressed (*co*), and short (*sh*), and the second chromosome dominant Bare (*Ba*), were mated to males of the same species heterozygous for Curly (*Cy*) associated with an inversion in the fourth chromosome. The F_1 daughters which showed *Ba* and *Cy* were selected and mated to *y sn v co sh* males. Among the progeny, only *y co sh Ba Cy* flies were selected and crossed to *D. persimilis* homozygous for a third chromosome mutant orange (*or*). The hybrids that showed *Ba* and *Cy* were heterozygous for the recessive mutant *y* located in the left limb of the X chromosome, *co* near the centromere, and *sh* in the right limb of the X chromosome, dominant *Ba* in the second chromosome, recessive *or* in the third chromosome, and dominant *Cy* in the fourth chromosome. The hybrids are heterozygous for five different inversions, one each in the right limb and in the left limb of the X chromosome, in the second chromosome, in the third chromosome, and in the fourth chromosome. The first four of the above inversions are the characteristic differences between the two species (TAN 1935), and the fifth inversion has been introduced with the Curly strain of *D. pseudoobscura*. The hybrids thus obtained were then backcrossed to *D. pseudoobscura* males carrying sex-linked recessives *y, sn, v, co, sh*, and the third chromosome recessive *or*.

According to the setting of the experiments, every one of the chromosomes of the hybrid females with the exception of the small fifth chromosome, in which no genes are available, is marked by one or more genes, which should make the different classes of the backcross progeny phenotypically distinguishable from each other. The 16 different classes of individuals each representing a specific combination of the four major pairs of chromosomes of the two species are shown schematically in the accompanying figure. They range from a type in which all chromosomes belong to *D. pseudoobscura* to the other extreme in which one chromosome of each pair comes from *D. pseudoobscura* and the other from *D. persimilis*. This, of course, leaves out an account of the crossing over that may occur despite the inversions and that escapes detection in these experiments.

Since different mutant strains may vary in sexual behavior, tests were made by comparing each class of backcross hybrid females with females of *D. pseudoobscura* carrying the corresponding mutants. For this purpose, mass cultures of the following four mutant stocks were made from which the 16 required classes of flies were obtained. One of these cultures contained *Ba* and *Cy;* it produces *Ba*, *Cy*, *Ba Cy*, and wild type. Another cross gave *Ba or*, *or Cy*,



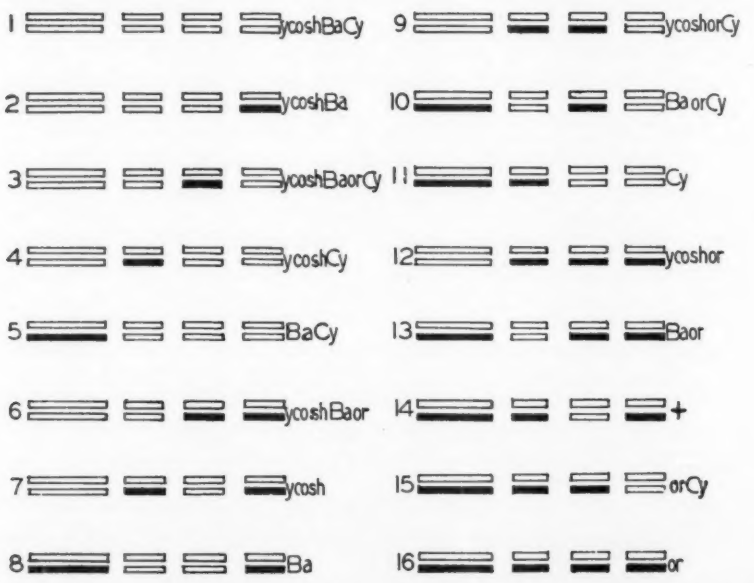


FIGURE 1. The chromosome constitution and the phenotype of the 16 different classes of females appearing in the backcross of the F_1 hybrid female heterozygous for yellow (*y*), compressed (*co*), short (*sh*), Bare (*Ba*), and Curly (*Cy*) to yellow (*y*), singed (*sn*), vermillion (*v*), compressed (*co*), short (*sh*), and orange (*or*) *D. pseudoobscura* males. *D. pseudoobscura* chromosomes—white; *D. persimilis chromosomes*—black.

or, and *Ba or Cy* flies. Still another cross furnished *y co sh Ba*, *y co sh Cy*, *y co sh*, and *y co sh Ba Cy*. Finally, a fourth culture gave rise to *y co sh Ba or*, *y co sh or Cy*, *y co sh or*, and *y co sh Ba or Cy* flies.

All flies used in these experiments were raised on a cream of wheat-molasses medium (SPASSKY 1943) at 22.5°C. In order to avoid crowding, the parent flies were transferred repeatedly to fresh culture bottles. When the adults in any culture began to emerge, the bottle was removed from the incubator and kept at room temperature. Newly emerged adults of both sexes were isolated every day and aged from five to seven days in separate vials with food at room temperature. In tests with *D. persimilis*, all flies were aged from seven to ten days, because they require a longer time to become sexually mature.

In the tests involving backcross hybrid progenies, it was frequently necessary to use flies with an age difference of as much as three days. In such cases, to minimize the possible effect of the age difference, control females with ages as heterogeneous as the hybrid females were used.

When two kinds of females involved in a given experiment are phenotypically alike, they are distinguished by clipping of wings, one type of females having the right and the other the left wing clipped. In flies carrying the *Cy* mutant, which may obscure the effect of wing clipping, a leg was clipped instead.

For each individual experiment, usually ten mature females of each of two kinds and five mature males of one kind were placed together in a vial containing food. Sometimes, when a sufficient number of properly aged virgin females of one or of both kinds was not available, the number of females used was reduced, but never to fewer than five of each kind. In these cases, the number of males used was also proportionately reduced, keeping the ratio of two females of each kind to one male. All mating experiments were carried on in an incubator of 25.5°C.

The length of time during which the females and males were kept together varied in individual cases, depending largely on which species of males were used. When *D. pseudoobscura* is used as male, two to three hours are usually sufficient to have more than 50 percent of the females inseminated. In the case of *D. persimilis* males, it takes sometimes even four or five days to reach a 50 percent insemination level. The experimental vials in which too few or too many (less than 10 percent, more than 90 percent) of the females were inseminated were discarded.

PREFERENTIAL MATING IN D. PSEUDOOBSCURA

Table 1 summarizes the results of the sexual preference tests between different strains of *D. pseudoobscura*. For each cross, the percentage of homogamically (matings among members of the same strain) and heterogamically (matings between members of different strains) inseminated females is given, together with the χ^2 which measures the statistical significance of the difference observed between these frequencies. The isolation index was originally proposed by STALKER (1942), and has since been used as a standard measure of the degree of randomness of mating. It is obtained by dividing the difference by the sum of the percentages of homogamic and heterogamic inseminations. If the matings are at random, the isolation index is 0 or close to it. If homo-

TABLE 1

Number of females dissected (N) and percentage carrying sperm (%) in different crosses of Drosophila pseudoobscura.

NO.	MALES	FEMALES	HOMOGAMIC		HETEROGAMIC		χ^2	INDEX
			N	%	N	%		
1	wild type	wild type, *or pr*	62	79.0	67	73.4	0.57	+0.04
2	wild type	wild type, *or*	43	58.1	41	90.2	11.07	−0.21
3	wild type	wild type, *ast*	38	76.3	39	66.7	0.91	+0.07
4	wild type	wild type, *w*	53	77.4	52	63.5	2.44	+0.10
5	wild type	wild type, *Ba Cy*	53	79.2	52	59.6	4.77	+0.14
6	wild type	wild type, *y sn v co sh*	49	69.4	53	79.2	1.29	−0.07
7	*or pr*	*or pr*, wild type	84	84.5	81	55.6	16.46	+0.20
8	*or pr*	*or pr*, *or*	34	41.2	36	44.4	0.08	−0.04
9	*or pr*	*or pr*, *ast*	19	78.9	19	57.9	1.96	+0.15
10	*or pr*	*or pr*, *w*	26	34.6	26	42.3	0.32	−0.10
11	*or pr*	*or pr*, *Ba Cy*	35	65.7	36	66.7	0.00	−0.01
12	*or pr*	*or pr*, *y sn v co sh*	29	79.3	27	74.1	0.19	+0.03
13	*or*	*or*, wild type	41	80.5	42	61.9	3.57	+0.12
14	*or*	*or*, *or pr*	43	60.5	45	77.8	3.07	−0.13
15	*or*	*or*, *ast*	37	86.5	37	37.8	11.66	+0.39
16	*or*	*or*, *w*	35	74.3	33	78.7	0.21	−0.03
17	*or*	*or*, *Ba Cy*	29	79.3	30	56.7	2.80	+0.17
18	*or*	*or*, *y sn v co sh*	76	46.1	76	57.9	2.14	−0.11
19	*ast*	*ast*, wild type	33	60.6	38	60.5	0.00	0.00
20	*ast*	*ast*, *or pr*	18	16.7	18	38.9	1.99	−0.40
21	*ast*	*ast*, *or*	39	53.8	35	80.0	3.00	−0.20
22	*ast*	*ast*, *w*	34	41.2	37	35.1	1.89	+0.08
23	*ast*	*ast*, *Ba Cy*	42	0.0	45	40.0	21.23	−1.00
24	*ast*	*ast*, *y sn v co sh*	38	60.5	39	66.7	1.23	−0.05
25	*w*	*w*, wild type	74	64.9	74	78.4	3.32	−0.09
26	*w*	*w*, *or pr*	27	37.0	27	11.1	4.98	+0.54
27	*w*	*w*, *or*	44	54.5	43	55.8	0.02	−0.01
28	*w*	*w*, *ast*	43	58.1	42	42.9	1.92	+0.15
29	*w*	*w*, *Ba Cy*	30	13.3	32	25.0	0.94	−0.30
30	*w*	*w*, *y sn v co sh*	55	36.4	54	37.0	0.01	−0.01
31	*Ba Cy*	*Ba Cy*, wild type	30	86.7	30	86.7	0.00	0.00
32	*Ba Cy*	*Ba Cy*, *or pr*	45	68.9	44	77.3	0.82	−0.06
33	*Ba Cy*	*Ba Cy*, *or*	29	79.3	29	96.6	4.00	−0.10
34	*Ba Cy*	*Ba Cy*, *ast*	33	81.6	35	51.4	9.57	+0.23
35	*Ba Cy*	*Ba Cy*, *w*	35	77.1	35	71.4	0.70	+0.04
36	*Ba Cy*	*Ba Cy*, *y sn v co sh*	28	82.1	30	76.7	0.57	+0.03
37	*y sn v co sh*	*y sn v co sh*, wild type	110	83.6	104	59.6	15.19	+0.17
38	*y sn v co sh*	*y sn v co sh*, *or pr*	57	66.7	61	36.1	10.99	+0.30
39	*y sn v co sh*	*y sn v co sh*, *or*	71	70.4	65	26.2	26.53	+0.46
40	*y sn v co sh*	*y sn v co sh*, *ast*	44	34.1	46	4.3	13.03	+0.78
41	*y sn v co sh*	*y sn v co sh*, *w*	64	35.9	66	7.6	15.45	+0.65
42	*y sn v co sh*	*y sn v co sh*, *Ba Cy*	45	37.8	46	26.1	1.48	+0.18

gamic or heterogamic matings are preferred, the index lies between 0 to +1, or between 0 and −1, respectively. An index +1 indicates complete isolation.

As shown in table 1, most of the crosses that involve wild type, *or pr*, *or*, *ast*, *w*, or *Ba Cy* males show no significant deviations from randomness of mating. The few exceptional cases are experiments Nos. 2, 7, 15, 23, and 34. Preferences for heterogamic inseminations are exhibited by wild type males in the crosses to *or* females (No. 2), and by *ast* males in the cross to *Ba Cy* females (No. 23). It is interesting to note that while *ast* males prefer *Ba Cy* females to their own, *Ba Cy* males prefer *Ba Cy* to *ast* females (No. 34). This relationship seems to hold true also for *or* and wild type. Wild type males mate with *or* more frequently than with wild type females (No. 2, I = −0.21), whereas *or* males prefer *or* to wild type females (No. 13, I = +0.12). Likewise, *or* males prefer *or* to *ast* females (No. 15, I = +0.39), while *ast* males seem to prefer *or* females to their own (No. 21, I = −0.20). This, however, is not true in the case of *or pr* and wild type flies: *or pr* males prefer *or pr* to wild type females (No. 7, I = +0.20), but wild type males mate at random with wild type and *or pr* females (No. 1, I = +0.04).

When yellow singed vermillion compressed short (*y sn v co sh*) flies are used as males, there is a strong preference for homogamic matings (Nos. 37 to 42). The isolation index varies in individual cases from +0.17 (No. 37) to +0.78 (No. 40). Statistically, all these values are significant with the exception of the case (No. 42) involving *Ba Cy* females. It is obvious that *y sn v co sh* males mate with females of their own strain more frequently than with females of other strains. The reciprocal crosses (Nos. 6, 12, 18, 24, 30, and 36), however, do not show any preference for either homogamic or heterogamic matings.

The mating preferences found in the cultures containing *y sn v co sh* females and males and wild type females (see above), may be caused directly by one of the mutant genes involved, or they may be due to genetic factors independent of these visible mutants. To clarify this point, *y sn v co sh* males were crossed to wild type females and the F_1 hybrid females were backcrossed to wild type males; *y sn v co sh* and *y* (crossover) males were selected in the progeny of the backcross. These males were crossed to their female sibs in small mass cultures, and in the following generation *y sn v co sh* and *y* strains were re-established. The sexual preferences of these "extracted" *y sn v co sh* and *y* flies were compared with those of the original *y sn v co sh* strain and of wild type and of *or* flies. The results are summarized in table 2. In this table, the designations of the "extracted" are underlined, and those of the original strain are shown in the usual type. It is evident that the behaviors of the original and the "extracted" *y sn v co sh* strains are alike within limits of experimental error. Furthermore, extracted *y* males inseminate more females of their own strain than that of wild type or *or* strains. Wild type males seem to show a slight preference for females of the "extracted" *y sn v co sh* and *y* strains in comparison to wild type females. These results are in accord with the

view that the peculiar mating preferences of the *y sn v co sh* strain are caused by the yellow mutant gene.

HYBRIDS OF D. PERSIMILIS AND D. PSEUDOOBSCURA

Tables 3 to 7 summarize the results of tests involving the pure species and different classes of backcross hybrids of *D. persimilis* and *D. pseudoobscura*. In the crosses involving hybrid females and males and females of the same species, the term "homogamic" refers to matings within the species and "heterogamic" to matings with hybrid females (Tables 3, 4, and 6). If the pure species females used belong to a species different from the male (Table 5 and 7), the genetic constitution of the hybrid females is closer to that of the males used than to that of the alien females. Hence, "homogamic" refers to matings with hybrid females and "heterogamic" to those with alien females.

TABLE 2

Number of females dissected (N) and percentage carrying sperm (%) in crosses with "extracted" y and y sn v co sh strains of Drosophila pseudoobscura.

NO.	MALES	FEMALES	HOMOGAMIC		HETEROGAMIC		χ^2	INDEX
			N	%	N	%		
1	*y sn v co sh*	*y sn v co sh*, wild type	72	76.4	62	58.1	4.96	+0.14
2	*y sn v co sh*	*y sn v co sh*, *or*	59	78.0	58	50.0	9.99	+0.22
3	*y sn v co sh*	*y sn v co sh*, *y sn v co sh*	32	46.9	32	40.6	0.21	+0.07
4	*y*	*y*, wild type	70	81.4	70	58.6	8.72	+0.16
5	*y*	*y*, *or*	135	72.6	134	59.0	5.24	+0.10
6	*y*	*y*, *y sn v co sh*	66	69.7	71	69.0	0.00	+0.01
7	wild type	wild type, *y sn v co sh*	25	44.0	27	77.8	6.60	−0.28
8	wild type	wild type, *y*	48	56.3	47	74.5	3.38	−0.14
9	*or*	*or*, *y sn v co sh*	32	46.9	34	52.9	0.24	−0.06
10	*or*	*or*, *y*	96	33.3	102	43.1	1.06	−0.13

The first experiment consisted in crossing *y co sh Ba Cy D. pseudoobscura* females to *or D. persimilis* males. The F_1 hybrid females were backcrossed to *y sn v co sh or D. pseudoobscura* males. The resulting backcross hybrid females have *D. pseudoobscura* cytoplasm, at least one complete set of *D. pseudoobscura* chromosomes, and from none to four *D. persimilis* chromosomes. The genetic composition of the different backcross females is symbolized in tables 3 to 7 by means of formulae in which the letter "a" stands for *D. pseudoobscura* and the letter "b" for *D. persimilis* chromosomes. Starting from the left, the first letter symbolizes the X chromosome, and the second, third, and fourth letters the second, third, and fourth chromosomes respectively. Thus, the formula $\frac{a}{b}\ \frac{a}{a}\ \frac{a}{b}\ \frac{a}{a}$ indicates a female having one *D. persimilis* X chromosome and one *D. persimilis* third chromosome, the other chromosomes being those of *D. pseudoobscura*. Altogether 16 classes of backcross females were tested

TABLE 3

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females (with pseudoobscura cytoplasm), D. pseudoobscura females, D. pseudoobscura males.

NO.	MALES *pseudo-obscura*	FEMALES *pseudoobscura*, HYBRIDS	HYBRID CHROMOSOMES				HOMO-GAMIC		HETERO-GAMIC		χ^2	INDEX
			X	II	III	IV	N	%	N	%		
1		*y co sh Ba Cy*	a/a	a/a	a/a	a/a	11	36.4	10	60.0	1.70	−0.24
2		*y co sh Ba*	a/a	a/a	a/a	a/b	15	40.0	16	56.3	0.72	−0.17
3		*y co sh Ba or Cy*	a/a	a/a	a/b	a/a	10	50.0	10	60.0	0.22	−0.09
4		*y co sh Cy*	a/a	a/b	a/a	a/a	23	43.5	23	65.2	2.04	−0.20
5		*Ba Cy*	a/b	a/a	a/a	a/a	28	64.3	29	72.4	0.32	−0.06
6		*y co sh Ba or*	a/a	a/a	a/b	a/b	16	31.3	16	75.0	6.18	−0.41
7		*y co sh*	a/a	a/b	a/a	a/b	30	60.0	30	76.7	1.78	−0.12
8		*Ba*	a/b	a/a	a/a	a/b	32	43.8	32	84.4	11.46	−0.32
9		*y co sh or Cy*	a/a	a/b	a/b	a/a	6	66.7	6	50.0	0.34	+0.14
10		*Ba or Cy*	a/b	a/a	a/b	a/a	34	58.8	33	72.7	1.54	−0.11
11		*Cy*	a/b	a/b	a/a	a/a	36	72.2	38	65.8	0.36	+0.05
12		*y co sh or*	a/a	a/b	a/b	a/b	20	60.0	20	60.0	0.00	0.00
13		*Ba or*	a/b	a/a	a/b	a/b	41	75.6	40	70.0	0.30	+0.04
14		+	a/b	a/b	a/a	a/b	34	67.6	34	79.4	2.10	−0.04
15		*or Cy*	a/b	a/b	a/b	a/a	30	83.3	32	75.0	0.66	+0.05
16		*or*	a/b	a/b	a/b	a/b	60	60.0	62	74.2	2.62	−0.11
Total							426	60.3	431	71.5	11.97	−0.08

(table 3). Each test consisted in placing together backcross females, *D. pseudo-obscura* females, and *D. pseudoobscura* males carrying the same visible mutants.

TABLE 4

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females (with persimilis cytoplasm), D. pseudoobscura females, and D. pseudoobscura males.

NO.	MALES *pseudo-obscura*	FEMALES *pseudoobscura*, HYBRIDS	HYBRID CHROMOSOMES X	II	III	IV	HOMO-GAMIC N	%	HETERO-GAMIC N	%	χ^2	INDEX
1		*y co sh Ba Cy*	a/a	a/a	a/a	a/a	10	30.0	9	22.2	0.18	+0.15
2		*y co sh Ba*	a/a	a/a	a/a	a/b	18	22.2	18	50.0	3.04	−0.39
3		*y co sh Ba or Cy*	a/a	a/a	a/b	a/a	7	28.6	9	22.2	0.06	+0.13
4		*y co sh Cy*	a/a	a/b	a/a	a/a	23	52.2	25	60.0	0.22	−0.07
5		*Ba Cy*	a/b	a/a	a/a	a/a	24	50.0	23	60.9	0.49	−0.10
6		*y co sh Ba or*	a/a	a/a	a/b	a/b	22	36.4	22	54.5	1.32	−0.20
7		*y co sh*	a/a	a/b	a/a	a/b	39	20.5	43	60.5	12.90	−0.49
8		*Ba*	a/b	a/a	a/a	a/b	28	39.3	29	34.5	0.10	+0.07
9		*y co sh or Cy*	a/a	a/b	a/b	a/a	54	40.7	57	63.2	5.37	−0.22
10		*Ba or Cy*	a/b	a/a	a/b	a/a	20	55.0	19	43.1	0.57	+0.13
11		*Cy*	a/b	a/b	a/a	a/a	30	63.3	33	75.8	1.20	−0.09
		y co sh or	a/a	a/b	a/b	a/b	35	42.9	38	71.1	6.30	−0.25
13		*Ba or*	a/b	a/a	a/b	a/a	39	61.5	38	76.3	0.00	−0.11
14		+	a/b	a/b	a/a	a/b	35	60.0	36	61.1	0.04	−0.01
15		*or Cy*	a/b	a/b	a/b	a/a	37	67.6	41	58.5	0.63	+0.07
16		*or*	a/b	a/b	a/b	a/b	47	51.1	47	59.6	0.62	−0.08
Total							468	47.2	487	69.3	14.06	−0.19

As shown in table 3, in most of the tests the matings took place either at random or else there was a slight preference for hybrid females.

The second experiment (table 4) paralleled the first, except that the original

TABLE 5

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females, D. pseudoobscura females, and D. persimilis males.

NO.	MALES *persimilis*	FEMALES *pseudoobscura*, HYBRIDS	HYBRID CHROMOSOMES				HETEROGAMIC ALIEN SP.		HOMOGAMIC HYBRID		χ^2	INDEX
			X	II	III	IV	N	%	N	%		
1	orange	*y co sh Ba Cy*	a/a	a/a	a/a	a/a	44	22.7	42	33.3	1.22	+0.19
2	orange	*y co sh Ba*	a/a	a/a	a/a	a/b	49	36.7	50	48.0	1.11	+0.13
3	orange	*y co sh Ba or Cy*	a/a	a/a	b/a	a/a	59	39.0	59	37.3	0.06	−0.02
4	orange	*y co ch sh Cy*	a/a	b/a	a/a	a/a	66	15.2	70	54.3	22.12	+0.56
5	orange	*Ba Cy*	a/b	a/a	a/a	a/a	50	22.0	49	57.1	12.24	+0.44
6	orange	*y co sh Ba or*	a/a	a/a	a/b	a/b	43	39.5	43	58.1	3.13	+0.19
7	orange	*y co sh*	a/a	b/a	a/a	a/b	56	12.5	57	57.9	24.58	+0.64
8	orange	*Ba*	a/b	a/a	a/a	a/b	62	12.9	63	34.9	8.34	+0.46
9	orange	*y co sh or Cy*	a/a	a/b	a/b	a/a	72	19.4	76	48.7	13.21	+0.43
10	orange	*Ba or Cy*	a/b	a/a	a/b	a/a	56	21.4	58	43.1	5.52	+0.34
11	orange	*Cy*	a/b	b/a	a/a	a/a	82	11.0	84	56.0	18.50	+0.67
12	orange	*y co sh or*	a/a	a/b	a/b	a/b	78	19.2	82	50.0	27.40	+0.45
13	orange	*Ba or*	a/b	a/a	a/b	a/b	59	16.9	61	44.3	10.77	+0.45
14	orange	+	a/b	a/b	a/a	a/b	99	30.3	100	53.0	10.79	+0.27
15	orange	*or Cy*	a/b	a/b	a/b	a/a	70	17.1	73	64.4	32.20	+0.58
16	orange	*or*	a/b	a/b	a/b	a/b	78	19.2	77	57.1	23.63	+0.50
Total							1023	21.6	1044	50.5	186.06	+0.42

cross involved *or D. persimilis* females and *y co sh Ba Cy D. pseudoobscura* males. Hence, the backcross hybrids had *D. persimilis* cytoplasm. The result obtained is the same as in the first experiment—namely, the mating is either

TABLE 6

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females, D. persimilis females, and D. persimilis males.

NO.	MALES *persimilis*	FEMALES *persimilis*, HYBRIDS	HYBRID CHROMOSOMES				HOMO-GAMIC		HETERO-GAMIC		χ^2	INDEX
			X	II	III	IV	N	%	N	%		
1	orange (*or*)	*y co sh Ba Cy*	a/a	a/a	a/a	a/a	18	50.0	17	41.2	0.22	+0.10
2	orange (*or*)	*y co sh Ba*	a/a	a/a	a/a	a/b	23	65.2	23	39.1	3.14	+0.25
3	orange (*or*)	*y co sh Ba or Cy*	a/a	a/a	a/b	a/a	16	50.0	17	17.6	3.09	+0.48
4	orange (*or*)	*y co sh Cy*	a/a	a/b	a/a	a/a	35	54.3	38	76.3	2.09	−0.17
5	orange (*or*)	*Ba Cy*	a/b	a/a	a/a	a/a	34	47.1	34	76.5	7.62	−0.24
6	orange (*or*)	*y co sh Ba or*	a/a	a/a	a/b	a/b	28	50.0	29	44.8	0.18	+0.05
7	orange (*or*)	*y co sh*	a/a	a/b	a/a	a/b	31	48.4	31	54.8	0.31	−0.06
8	orange (*or*)	*Ba*	a/b	a/a	a/a	a/b	26	42.3	29	34.5	0.37	+0.10
9	orange (*or*)	*y co sh or Cy*	a/a	a/b	a/b	a/a	30	56.7	30	66.7	0.73	−0.08
10	orange (*or*)	*Ba or Cy*	a/b	a/a	a/b	a/a	13	69.2	14	42.9	1.57	+0.23
11	orange (*or*)	*Cy*	a/b	a/b	a/a	a/a	27	33.3	32	56.3	3.19	−0.26
12	orange (*or*)	*y co sh or*	a/a	a/b	a/b	a/b	30	50.0	32	62.5	0.84	−0.11
13	orange (*or*)	*Ba or*	a/b	a/a	a/b	a/b	25	36.0	25	28.0	0.38	+0.13
14	orange (*or*)	+	a/b	a/b	a/a	a/b	38	34.2	39	51.3	2.31	−0.20
15	orange (*or*)	*or Cy*	a/b	a/b	a/b	a/a	40	42.5	45	62.2	3.34	−0.19
16	orange (*or*)	*or*	a/b	a/b	a/b	a/b	47	29.8	47	55.3	5.82	−0.30
Total							461	45.6	482	53.7	6.14	−0.08

random or else there is a slight preference for insemination of hybrid females. No striking differences in the behavior of different classes of hybrids is apparent.

The third experiment (table 5) differed from the first in that orange mutant males of *D. persimilis* were used in the final test instead of *D. pseudoobscura* males. Hence, in this experiment orange *D. persimilis* males had the choice of mating with pure *D. pseudoobscura* females and with hybrid females having *D. pseudoobscura* cytoplasm, from one to two complete sets of *D. pseudoobscura* chromosomes, and from none to four *D. persimilis* chromosomes. The result obtained is quite clear (table 5). Namely, *D. persimilis* males mate with hybrid females in preference to the pure *D. pseudoobscura* females; this is found in all the tests except Nos. 1, 2, 3, and 6. The four exceptional tests are those in which the backcross hybrid females carried only *D. pseudoobscura* chromo-

TABLE 7

Number of females dissected (N) and percentage carrying sperm (%) in crosses between backcross hybrid females, D. persimilis females, and D. pseudoobscura males.

NO.	MALES *pseudo-obscura*	FEMALES *persimilis*, HYBRIDS		HYBRID CHROMOSOMES				HOMO-GAMIC HYBRID		HETERO-GAMIC ALIEN SP.		χ^2	INDEX
				X	II	III	IV	N	%	N	%		
1	*Ba*	*or*	*Ba*	a/b	a/a	a/a	a/b	14	78.6	12	0.0	16.49	+1.00
2	*Cy*	*or*	*Cy*	a/b	a/b	a/a	a/a	15	100.0	17	11.8	24.70	+0.79
3	*Ba or*	*or*	*Ba or*	a/b	a/a	a/b	a/b	7	57.1	5	0.0	4.56	+1.00
4	+	*or*	+	a/b	a/b	a/a	a/b	34	97.1	34	8.8	53.12	+0.83
5	*or Cy*	*or*	*or Cy*	a/b	a/b	a/b	a/a	16	100.0	17	11.8	26.08	+0.79
6	*or*	*or*	*or*	a/b	a/b	a/b	a/b	29	82.6	27	14.8	25.84	+0.70
Total								115	89.6	112	9.8	144.00	+0.80

somes (No. 1), or a fourth chromosome of *D. persimilis* (No. 2), or a third chromosome of *D. persimilis* (No. 3), or a third and a fourth chromosome of *D. persimilis* (No. 6).

In the fourth experiment, *y co sh Ba Cy* females of *D. pseudoobscura* were crossed to *or* males of *D. persimilis*. The F_1 hybrid females were backcrossed to *y sn v co sh or D. pseudoobscura* males. The resulting backcross hybrids had, therefore, *D. pseudoobscura* cytoplasm, from one to two sets of *D. pseudoobscura* chromosomes, and from one set to no *D. persimilis* chromosomes. Such backcross hybrid females were placed in vials with orange *D. persimilis* females and orange *D. persimilis* males (table 6). Consequently, the *D. persimilis* males had the choice of pure *D. persimilis* females and of females which

were predominantly *D. pseudoobscura* but which had some *D. persimilis* chromosomes. The observed matings mostly failed to deviate significantly from randomness. However, there seems to be a slight tendency for homogamic mating in the tests (Nos. 1, 2, and 3) in which the hybrid females had either none or only one third or fourth chromosome from *D. persimilis*.

The fifth experiment started like the fourth (see above), but the backcross hybrid females were tested with orange *D. persimilis* females and with *D. pseudoobscura* males carrying the same mutant genes as the hybrid females in the same vial. The results (table 7) show a striking preference for mating with hybrid females.

DISCUSSION

Among the results obtained in the intraspecific sexual preference tests, the behavior of the mutant yellow deserves some consideration. Whenever males carrying yellow, either alone or in combination with other mutant genes, are placed with a mixture of yellow and non-yellow females, there is always a preference for homogamic matings. Experiments with outcrossing and "extracting" yellow (table 2) indicate that the above behavior is caused by the change at the yellow locus rather than by associated modifying genes. Furthermore, this behavior of yellow is not confined to *Drosophila pseudoobscura* but occurs also in other species in which apparently homologous mutants are known and have been tested for sexual preference—namely, in *D. melanogaster* (SPETT 1931; NIKORO, GUSSEV, PAVLOV, and GRIASNOV 1935) and in *D. suboscura* (RENDEL 1945). The causes of this peculiar behavior of the yellow mutant are not clear. According to MAYR (1946), mating preferences may be controlled by specific attractions, degree of activity, and by physical compatibility of the genitalia. Yellow flies are hardly more active sexually than the non-yellow ones, since STURTEVANT (1915) and DIEDERICH (1941) reported that in *D. melanogaster*, yellow and yellow white males copulate with fewer females than the normal males when the two kinds of males are mated to yellow or yellow white females. RENDEL (1945) found that in *D. subobscura*, yellow males are discriminated against in mating by females of some, but not of all, wild type strains. The chitinous genitalia of yellow and non-yellow males seem to be morphologically similar. A specific attraction between yellow females and males, or a repulsion between yellow males and non-yellow females, may be present, but it must be noted that when non-yellow males are placed with yellow and non-yellow females, the matings are usually at random.

MAYR (1946) found that when *D. persimilis* males have a choice of mating with females of their own species and with F_1 hybrid females from the cross *D. persimilis*×*D. pseudoobscura*, more hybrids than conspecific females are inseminated. When *D. pseudoobscura* males are offered a choice of their own and of F_1 hybrid females, there is a slight preference for homogamic matings; but this preference is much less pronounced than that found when *D. pseudoobscura* males have a choice of *D. pseudoobscura* and *D. persimilis* females. MAYR attributes these results to the F_1 hybrid females being more vigorous and active than females of the pure species, presumably on account of hetero-

sis. This explanation does not fit our data. Males of *D. pseudoobscura* mate with females of their own species and with backcross hybrid females either at random or else show a slight preference for the hybrids (tables 3 and 4). The backcross hybrid females range in genetic constitution from almost pure *D. pseudoobscura* (class No. 1) to a class having equal number of chromosomes of *D. pseudoobscura* and *D. persimilis* (class No. 16). In contrast to the F_1 hybrid females, which are at least as vigorous as those of the pure species, the backcross hybrid females exhibit every sign of constitutional weakness (DOBZHANSKY 1936; DOBZHANSKY and EPLING 1944). The failure of *D. pseudoobscura* males to discriminate against hybrid females carrying from one to four *D. persimilis* chromosomes (classes Nos. 2 to 16 in tables 3 and 4) suggests, then, a dominance of the *D. pseudoobscura* genes which cause a female to be acceptable to a *D. pseudoobscura* male. Indeed, all the backcross hybrid females in these experiments carried at least one full set of *D. pseudoobscura* chromosomes (fig. 1).

Much clearer are the results obtained when *D. persimilis* males are placed together with mixtures of backcross hybrid females and pure *D. pseudoobscura* females (table 5). The backcross hybrid females in these experiments represent the same range of genetic constitutions as in the experiments dealt with above (fig. 1). Now, *D. persimilis* males definitely prefer backcross hybrid females having whole sets of the chromosomes of both parental species (class No. 16) to pure *D. pseudoobscura* females. On the other hand, backcross hybrid females which have only, or mainly, *D. pseudoobscura* chromosomes (class No. 1) are no more acceptable to *D. persimilis* males than are pure *D. pseudoobscura* females. A more detailed analysis of the data in table 5 discloses that the genes responsible for the acceptability of females to *D. persimilis* males seem to be dominant and to lie in probably all chromosomes, with X and second chromosomes being more important than the third and the fourth. Thus, females having one second chromosome of *D. persimilis*, and the remainder of the chromosomes from *D. pseudoobscura* (class No. 4), are definitely more acceptable to *D. persimilis* males than are pure *D. pseudoobscura* females. The same holds for females having a single *D. persimilis* X chromosome in an otherwise pure *D. pseudoobscura* complex (class No. 5).

Essentially the same conclusions follow from the experiments in which *D. persimilis* males had to discriminate between backcross hybrid females and pure *D. persimilis* females (table 6). Here the backcross hybrids having equal numbers of chromosomes of the two species (class No. 16) are preferred to pure conspecific females. This may seem paradoxical, but this result agrees with those of MAYR (1946), who, as mentioned above, found that F_1 hybrid females are inseminated by *D. persimilis* males more often than are pure *D. persimilis* females. On the other hand, backcross hybrids having only *D. pseudoobscura* chromosomes (class No. 1), or a single fourth or third chromosome of *D. persimilis* in an otherwise pure *D. pseudoobscura* chromosome complex (classes Nos. 2 and 3), are discriminated against in favor of pure *D. persimilis* females. The X and second chromosomes seem to be most important.

The data at hand therefore support the view that the sexual isolation between *D. pseudoobscura* and *D. persimilis* is caused by a system of at least partly dominant factors carried by each species. *D. pseudoobscura* may be assumed to carry the gene complexes *TT*, and *D. persimilis* the corresponding, but nonallelic, gene groups *SS;* the F_1 hybrids between these species are, then, *TtSs*. The genetic mechanism of sexual isolation may thus be similar to that producing the sterility of the hybrids between them (DOBZHANSKY 1936). In both cases the gene divergence has arisen presumably during the stage when *D. pseudoobscura* and *D. persimilis* were geographically isolated races, and it became accentuated and built up to form reproductive isolating mechanisms when the stage of speciation was reached. The most important difference appears to be that, whereas the sterility genes bring about a complete sterility of F_1 hybrid males, the genes responsible for the sexual attraction are dominant and make the F_1 hybrid females acceptable to males of either pure species. From the standpoint of maintenance of the separation of the two species in nature, the mechanism of sexual isolation is thus insecure. Nevertheless, it probably serves well its biological function as a part of a system of isolating mechanisms.

SUMMARY

Sexual preference tests were made using different mutant strains of *Drosophila pseudoobscura*—namely, orange purple (*or pr*), orange (*or*), white (*w*), aristapedia (*ast*), Bare Curly (*Ba Cy*), yellow singed vermillion compressed short (*y sn v co sh*), and one wild type strain. The *y sn v co sh* males inseminate more females of their own kind than females of other mutant strains or of wild type. This effect depends upon the mutant yellow. Other tests showing preferential matings include: (1) wild type males inseminate more *or* than wild type females, (2) *or pr* males inseminate more *or pr* than wild type females, (3) *ast* males inseminate more *Ba Cy* than *ast* females, (4) *Ba Cy* males inseminate more *Ba Cy* than *ast* females and (5) *or* males inseminate more *or* than *ast* females.

Hybrids of *D. pseudoobscura* and *D. persimilis* were obtained ranging in chromosomal constitution from individuals having all chromosomes of *D. pseudoobscura* to individuals having equal numbers of *D. pseudoobscura* and *D. persimilis* chromosomes. When representatives of the different classes of backcross hybrid females were placed together with *D. pseudoobscura* females and *D. pseudoobscura* males, slightly more hybrid than *D. pseudoobscura* females were inseminated.

When a mixture of backcross hybrid females and *D. persimilis* females are exposed to *D. persimilis* males, the results also show a slight preference for mating with the hybrids. But those classes of hybrid females which do not carry the X chromosome, or the second chromosome, of *D. persimilis* are preferred by *D. persimilis* males to a smaller extent than those females which carry these chromosomes.

When *D. persimilis* males are placed with *D. pseudoobscura* females and with backcross hybrid females, the results show a significant preference for mating

with the hybrids, with the exception of the four classes of hybrids which do not carry either an X chromosome or a second chromosome of *D. persimilis*. This suggests that the main factors which distinguish the mating behavior of *D. persimilis* from *D. pseudoobscura* lie in the X and second chromosomes.

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DELETERIOUS RECESSIVE GENES IN DAIRY BULLS SELECTED AT RANDOM[1]

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INTRODUCTION

THE genetics of farm mammals have not been investigated in a manner comparable to the intensive studies made, during the last forty years, with Drosophila, laboratory rodents, and chickens. Nevertheless, a large number of mutant genes have been reported in farm mammals. These mutants have been discovered more or less by chance when sufficient inbreeding has brought them to light. Many more have probably occurred that were not reported. Several investigators—HUTT (1934), EATON (1937), MOHR (1939), and others—have compiled these mutants from time to time. LERNER (1944) has made the most recent compilation. His check lists for cattle, horses, swine, sheep, and chickens show that cattle lead, with 25 lethals. Since, however, there are two recognizable phenotypes of recessive achondroplasia, the number is 26. The undesirable nonlethals reported are probably more numerous, but there is no compilation of them.

This paper will summarize the occurrence of the undesirable recessive genes that were found in the original sires tested in the California dairy cattle breeding experiment. It is realized that the data are insufficient to establish gene frequency values, mutation rates, and other related values pertinent to the dairy cattle population as a whole. Perhaps this report will stimulate other investigators with similar data to publish, so that eventually sufficient data will be amassed to permit a complete study of gene frequencies and related phenomena in cattle.

BACKGROUND OF THE EXPERIMENT

The breeding project was started by one of us (REGAN) with Jersey cattle in 1918 at the NEW JERSEY AGRICULTURAL EXPERIMENT STATION. Another one of us (MEAD) became associated with the study two years later. The plan was to progeny-test several unrelated sires for their ability to transmit milk and fat production. In order to fix production in a more homozygous state, sire-daughter matings were to be employed whenever possible. Several inbred lines were to be established. It was hoped that one or more of the bulls progeny tested would uniformly transmit high yields of milk and fat. The primary objective was to study the inheritance of milk and fat yields within inbred lines and in crosses between the inbred lines that were to be established.

In 1923, after the work had been under way five years, the whole experi-

[1] This work was supported by the Kellogg Fund of the UNIVERSITY OF CALIFORNIA for the application of genetics to farm animals.

ment, including the cattle, was moved to the UNIVERSITY OF CALIFORNIA, when REGAN and MEAD joined the staff of the College of Agriculture here. The Jersey herd already established at the California station was incorporated into the experiment, so that the number of animals and inbred families was augmented. After the Jersey program was well under way, sire-daughter matings were employed in the smaller Holstein herd of the California station. Thus far, one Holstein bull has been tested for recessives.

From the beginning of the experiment, feeding and management have closely conformed to the accepted practices of good dairy husbandry. Since 1928, except for a small outbreak in 1936 and 1937, the herd has been free from Brucella infection. All heifers classed as sterile reacted negatively to the agglutination test for Brucellosis. Trichomoniasis has never been suspected or found in any of the sires used, and apparently none of the females has been infected. Throughout, there has been continuity of supervision and close attention to details by REGAN and MEAD.

TABLE 1

Identity of bulls tested.

BREED	HERD NUMBER	REGISTERED NAME	REGISTRATION NUMBER	INBREEDING COEFFICIENT
Jersey	3000	Lucky Fern	126758	0.02
Jersey	300	Octavia's Rinda Lad	188257	0.01
Jersey	300A	Rinda Lad's St. Mawes Lad	201213	0.13
Jersey	300C	Gravity's Exile of Lusscroft	162551	0.03
Jersey	300D	Pogis Torono Experimenter	177266	0.06
Holstein	100B	Bear Valley Ormsby Esther	518683	0.00

THE STOCKS

Since the establishment of high-producing families was the primary object, sires were carefully selected upon evidence of high production as found in their pedigrees. The pedigrees of some were more promising than others, but all manifested better than average potentialities for production. The original sires selected came from different herds, and none had common ancestry in the first four generations. Thus they were considered to be unrelated. Several generations farther back, however, the Jersey sires traced to a few animals that were common to them all.

Sire 3000 was in use at the New Jersey station when the experiment was planned, and he and his progeny were made a part of the experiment. Sire 300 was in service at Davis when the experiment was moved to California. All the other sires were selected specifically for the breeding project.

When sire 300 died prematurely, a paternal half brother (300A) was brought in as a replacement. Table 1 gives the identity of all the sires tested and their inbreeding coefficients (WRIGHT 1922). The coefficients are low. Those shown for both 3000 and 300 were obtained by including several long chains with

Golden Lad (E.H.B. 1242) as the common ancestor. Ordinarily, the coefficients for these two animals would be counted as 0. The most highly inbred bull (300A) resulted from a sire-granddaughter mating.

The foundation cows to which the sires were mated came from two sources, the New Jersey and the California agricultural experiment stations. The two cow herds were not closely related. In each herd the foundation cows were sired by several different bulls. Inbreeding coefficients of the foundation cows were not calculated, but all were low or 0.

Deleterious recessive genes were never considered in the selection of sires or dams. In fact, dominant achondroplasia was the only lethal mutant that had been reported when the experiment was planned. So far as deleterious genes are concerned, all the original sires as well as the foundation cows to which they were mated were selected at random. The present paper does not consider the occurrence or frequency of the genes in the descendants of the foundation stock, since the authors have already presented these data in several papers.

Eight different autosomal recessive genes were found, each conditioning a specific type of anomaly. The lethals are epitheliogenesis imperfecta (REGAN, MEAD, and GREGORY 1935) and a recessive type involving achondroplasia-like symptoms (GREGORY, MEAD, and REGAN 1942), although some have questioned whether the achondroplasia is genuine. The nonlethals are congenital cataract (GREGORY, MEAD, and REGAN 1943), proportionate dwarfism, and flexed pasterns (MEAD, GREGORY, and REGAN 1942, 1943), strabismus (REGAN, MEAD, GREGORY 1944), and two different genes for female sterility GREGORY, REGAN, and MEAD 1945).

TESTING OF SIRES AND RESULTS

The sire-daughter mating, which was used whenever possible, is the most practical and efficient test for recessive genes in cattle. All the matings employed, however, that gave positive information on the genotype of a sire were utilized. If a bull died before extensive sire-daughter matings could be made, then either a son mated to paternal half sisters or later descendants of the sire were used to complete the test. It is probable that all the bulls were not adequately tested and that some of the recessive genes escaped detection. There can be no doubt, however, concerning the genes that were positively identified. Since details of the specific tests, with proof of each gene's identity, are given in our other papers cited, a repetition here is unnecessary. Table 2 summarizes information concerning the time the bulls were used, the total progeny, the progeny resulting from sire-daughter matings, and the type of test used to prove each bull. All final dates refer to the death of the sire. Bulls proved by sire-daughter matings were proved "directly"; those proved through sons, "indirectly."

Sires 3000, 300, and 300A died before direct tests could be made. None of their offspring manifested any recessive defects whatsoever. All were proved through sons or grandsons by indirect tests. The results obtained from these matings are summarized in table 3. Columns 2 and 3 show the herd number of

the replacement sires used and the relation to the original sire. The test progeny shown in columns 4 and 5 are not all of equal value in proving the original sire, since some of the matings should give a higher percentage of recessive combinations than others. A minus (—) check in the remaining six columns indicates that the specific defect did not occur in the test progeny, whereas a number indicates the recessive segregates that did occur.

Sire 3000 was tested through a son, 320A, and two grandsons, 433A and

TABLE 2

Summary of information regarding the testing of the sires.

SIRE	DATES USED		TOTAL PROGENY		PROGENY FROM SIRE-DAUGHTER MATINGS		METHOD OF PROVING
			MALE	FEMALE	MALE	FEMALE	
3000	8- 1-16	2-14-22	19	18	1	4	Indirect
300	3-28-21	9-18-25	9	18	3	2	Indirect
300A	12-20-28	8-17-33	21	10	0	0	Indirect
300C	4-25-20	1-19-32	14	25	8	11	Direct & indirect
300D	5-15-20	7-10-32	70	65	17	23	Direct
100B	3-10-28	3-26-41	111	98	58	58	Direct

TABLE 3

Sires tested indirectly through sons or grandsons.

ORIGINAL SIRES	REPLACEMENT SIRES AND RELATION TO ORIGINAL		TOTAL PROGENY FROM TEST MATINGS		EPITHELIO-GENESIS IMPER-FECTA	ACHON-DRO-PLASIA	CATA-RACT	STRA-BISMUS	FLEXED PAS-TERNS	FEMALE STERIL-ITY
			MALE	FEMALE						
3000	320A	Son	23	25	—	1	—	3	1	—
	433A	Son of 320A	40	29	—	8	—	—	8	—
	428A	Son of 320A	6	6	—	—	—	1	—	—
300	375A	Son	0	4	—	—	—	—	—	—
	333C	Son	27	19	—	—	11	—	—	2
	370A*	Grandson	140	97	—	—	—	—	—	5
300A	386A†	Son	16	20	5	—	1	—	3	—
	403A	Son	9	11	1	—	—	—	—	—

* Son of 320A, and maternal grandson of 300.
† Also a maternal grandson of 300.

428A, both of which were sons of 320A, produced by sire-daughter matings. Bulls 320A and 433A were rather completely tested, but the test for 428A is incomplete. These results and the pedigree analysis in earlier papers already cited offer irrefutable proof that 3000 is heterozygous for the genes which condition recessive achondroplasia, strabismus, and flexed pasterns. Bull 320A is also heterozygous for all three; 433A for achondroplasia and flexed pasterns only; 428A was positively proved for strabismus, but his status as to achondro-

plasia and flexed pasterns was not determined with absolute certainty (see footnote on 428A, table 8).

Sire 300 was proved through a son and a grandson. The first replacement son used, 375A, died early without yielding any positive information. Later another son 333C, gave absolute proof on both cataract and female sterility. Bull 370A, a grandson of both 300 and 3000, was used extensively in test matings, many of which were sire-daughter. He proved to be free from all the undesirable recessives except female sterility.

Sire 300A, a paternal half brother of 300, is really a replacement sire brought in to substitute for 375A before 333C was used. When mated to daughters of 300 and 375A, he did not produce any animals that were cataractous or female sterile. At his death he was replaced by a son, 386A, who was also a maternal grandson of 300. The tests proved that both 386A and his sire, 300A, were

TABLE 4

Summary of the recessive genes carried by six sires selected at random.

SIRE	EPITHELIO-GENESIS IMPERFECTA	RECESSIVE ACHONDRO-PLASIA	PROPOR-TIONATE DWARFISM	CONGENI-TAL CATARACT	STRABIS-MUS	FLEXED PASTERNS	FEMALE STERILITY
3000	+	+	−	−	+	+	−
300	−	−	−	+	−	−	+
300A	+	−	−	−	−	+	−
300C	−	−	+	−	−	−	+
300D	−	−	−	−	+	−	++
100B	−	−	−	−	−	−	+

− = Defect did not appear in test progeny, assumed to be homozygous normal.
+ = Heterozygous for specific recessive gene.
++ = Homozygous for specific recessive gene.

heterozygous for epitheliogenesis imperfecta and flexed pasterns. Bull 386A was also found heterozygous for congenital cataract, which he inherited from 300. Bull 403A, a son of 300A, was heterozygous for epitheliogenesis imperfecta, but was not completely proved for the other recessives that he might possess. Even though 300 and 300A are paternal half brothers, the undesirable recessive genes that each carried were not common to both of them.

Bulls 300D and 100B were tested directly by sire-daughter matings. Bull 300C was tested directly for proportionate dwarfism and indirectly for female sterility. Table 4 summarizes the results of all tests, direct and indirect. Bull 300D produced a large number of progeny and a fair number from sire-daughter matings (table 2). The mating test indicates that he is homozygous for female sterility. The improbability that a homozygous animal should be found in such a small sample is recognized. However, 300D has a low inbreeding coefficient; and this gives a slight advantage favoring the possibility of homozygosity. Evidence from the University herd, ALBRECHTSEN (1917), ECKLES (1929), and cooperating herd L (table 5) definitely indicates that

homozygosity for sterility is not uncommon. Before University sires were placed in herd L the preceding sire, which was unrelated to University stock, produced at least one sterile daughter when used in sire-daughter matings. Later when two University sires, descendants of 300 and 300D respectively, were used in the herd, one heifer out of eight proved to be sterile. When all these contingencies are considered, it seems safe to assume that 300D is homozygous for female sterility.

The most satisfactory test of all was that of the Holstein sire, 100B, He produced over 200 offspring, 116 of which were from sire-daughter matings

TABLE 5

Summary of mutant genes found in cows unrelated to the six sires tested.

HERD	MUTANT				
	DWARFISM	CATARACT	STRABISMUS	FLEXED PASTERNS	FEMALE STERILITY
UNIVERSITY OF CALIFORNIA	2 (349,406)*		1 (386)		3 (419, 491, 1015)
Cooperating Herds					
A				1 (son of 164)	
D					1 (32)
F		1 (40)	1 (632)		
H		1 (283)			
J					2 (92, 197)
K					1 (111)
L			1 (19)		5 (20, 25, 42, 57, 75)
N			1 (Velda)		

* The first number represents the number of cows proved, while the numbers in parentheses refer to the herd identity of the offspring that proved them.

(table 2). The only recessive he proved to carry was a gene for female sterility. The test on 300C was the least satisfactory made on any of the sires. He had few test progeny (table 2); and no tests were made in later generations, because all his descendants were discarded on account of low production of milk and fat. He produced four dwarf daughters, two from unrelated females and two from sire-daughter matings. The dwarfness was certainly hereditary, and one is justified in concluding that 300C is heterozygous for a recessive gene that conditions dwarfness. The test for female sterility, although less conclusive, is considered valid. When mated to a granddaughter of 300D, bull 300C produced one sterile daughter. He produced only five daughters from sire-daughter matings that lived to a sufficient age to determine their genotype, and all proved fertile. The only chance for error in determining his genotype for female sterility is that some unknown adverse environmental condition might have caused the heifer in question to be sterile. All the tests in the herd yield some information on the relative importance of heredity and environment in causing sterility. Twenty heifers in the herd were definitely sterile. Of

these, 19 were sired by bulls that were either heterozygous or homozygous for a gene that conditions sterility in heifers. Thus 95 percent of the infertile heifers can be accounted for by heredity. Since heredity is responsible for the high percentage of sterility in the herd and since the sterile daughter of 300C is also a granddaughter of 300D, a bull apparently homozygous for the conditioning gene, the logical conclusion is that 300C is heterozygous. All the tests and evidence indicate that the Jersey sires 300, 300C and 300D may possess the same gene for female sterility.

Since the Holsteins and Jerseys were never crossed, no mating tests are available to determine the relation of the sterility found in the two breeds. All the sterile Jerseys manifested regular heat periods, whereas, with but two exceptions in which one of the heifers exhibited nymphomania, the sterile Holsteins failed to manifest heat. One might consider it an open question whether the female sterility observed in the Jerseys and Holsteins is caused by the same or by different genes. The differential behavior in the expression of heat periods however, supports the conclusion that two different genes are involved. If more details of the tests or anomalies are desired, our papers cited earlier may be consulted.

There were no planned tests to study recessive genes in the foundation cows. Since the average dairy cow produces slightly less than four calves, individual tests would usually be incomplete. Techniques for this type of analysis, however, have been developed and are employed primarily in the study of genetics in human populations. It happened that six foundation cows were proved by the original sires to be heterozygous for three of the genes studied; the data are assembled in table 5. Further information on some of these genes in unrelated stock was obtained from cooperating diarymen who were using UNIVERSITY OF CALIFORNIA Jersey bulls descended from the sires tested. The gene that conditions congenital cataract was found in two herds, and the strabismus gene in three. Flexed pasterns certainly appears in at least one herd, and probably in others. Most dairymen have seen calves born with flexed pasterns and know from experience that the afflicted calves will out grow the condition and become normal. Such calves therefore evoke little interest or comment.

Sterile heifers were found in eight of the cooperating dairy herds. In four of the herds, both the sire and the dam of each sterile female were descendants of 300 or 300D. The other four herds produced nine sterile heifers. With but two exceptions all were sired by descendants of 300 or 300D (table 5). The dams of these heifers were not descended from any of the six sires tested. Thus five of the seven defects found in the six bulls were also found in unrelated foundation cows to which they were mated, or in unrelated cows mated to sons or grandsons of the six tested sires.

The frequency values of the mutant genes found in the six sires tested are assembled in table 6. The tested sample is so limited that the values found cannot be considered as representative of the cattle population as a whole. All the values found are evidently much higher than those of the general popula-

tion; but they may indicate, to some extent, the relative frequency of the latter.

Table 6 indicates the value of selection pressures of the mutants in the homozygous state, if determined. Since all the mutants are completely recessive, there is complete protection against selection in the heterozygotes. Although the exact value was not determined, there is some positive selection pressure against the homozygous classes of proportionate dwarfism, cataract, and strabismus; but the actual values, though not determined, are relatively low. The proportionate dwarfism found in Jerseys overlaps with the small

TABLE 6

The values of the gene frequencies found in the six sires tested and the selection pressure.

GENE	GENE FREQUENCY (1-q)	SELECTION PRESSURE s
Epithelogenesis Imperfecta	0.083	1.0
Recessive achondroplasia	0.083	1.0
Dwarfism	0.083	+
Cataract	0.083	+
Strabismus	0.167	+ slight
Flexed pasterns	0.167	+ slight
Female sterility (1) Normal estrus found in Jerseys only, "Jersey type"	0.333	0.5
Female sterility (2) Abnormal estrus found in Holsteins only, "Holstein type"	0.083	0.5

refined type highly prized by some breeders primarily interested in the show ring. Hence this type of dwarfism may actually be favored in certain purebred herds where the major emphasis is placed upon show-ring competition. On the other hand, there is strong selection against this type of dwarfism in the herds that favor large cows. Cataract, even though congenital, varies in its effect upon vision. Many of the afflicted animals competed well with normal members of the herd, whereas a smaller percentage could not. The selection pressure (s) is probably close to 0.25. Strabismus does not become manifest until the animal is about a year old; and the condition is progressive with age. The most advanced and noticeable stage is usually not reached before five or six years, or even later. The selection pressure is therefore low or negligible. In the wild state the pressure against flexed pasterns would be considerable, probably approaching 0.5 or even more. Since afflicted calves soon become normal, the defect is of no consequence if, during the first few days after birth, they are given the necessary aid in nursing.

Although there is complete selection against cows homozygous for female sterility, the fertility of the heterozygote seems not to be impaired in any way. It is assumed that bulls homozgyous for female sterility are fertile. When both sexes are considered, the selection pressure against female sterility is 0.5.

Judging from the studies in the UNIVERSITY OF CALIFORNIA herd and the herds of cooperating diarymen, the genes conditioning female sterility have a much higher frequency than any of the other genes found. It is assumed that two different independent recessive genes, the "Jersey" and "Holstein" respectively, are responsible and that either alone can cause the condition to become manifest. Table 7 gives the estimated occurrence of sterile heifers, based upon the value of the gene frequency found. The square of the gene frequency $(1-q)^2$ gives the proportion of sterile females exepected in a large population mating at random (HARDY 1908). If there are two independent genes, each causing sterility, the frequency of the one that allows the heat periods to be

TABLE 7

The expected amount of sterility from two independent genes each of which conditions sterility, based upon the frequency found in sires compared with the actual sterility observed in the University of California herd and by other investigators.

	FREQUENCY (1-q)	EXPECTED OR OBSERVED STERILITY	PROPORTION OF STERILE TO TOTAL
Two independent genes			
"Jersey" gene	0.333		
"Holstein" gene	0.083	0.1149*	1:9
Actual sterility found in University of California herd		0.057	1:17.5
Sterility observed by other investigators			
ALBRECHTSEN		0.048	1:21
ECKLES		0.074	1:13.5

* Calculated from the formula $[q_A A+(1-qA)a]^2[q_B B+(1-qB)b]^2$.

normal (Jersey type) is 0.333, and the expected proportion of sterility from this gene alone in a population mating at random would be 0.111. The gene in which the heat period is abnormal (Holstein type) has a frequency of 0.083, and the expected proportion of sterility from it would be 0.0069. When both genes are segregating together, in a population mating at random, the proportion of sterility should be 0.115 or one sterile heifer in nine (table 7).

WRIGHT (private communication) has pointed out that if the frequency of the female sterile gene found in the Jersey breed is 0.33 and there is no special type of selection that favors the gene in the heterozygous state, it would require a mutation rate of 0.05 to maintain the gene at the frequency observed.

These estimates on the proportion of sterility expected in a population mating at random, based upon the gene frequencies observed in the sires tested, can be checked against the incidence of sterility in heifers as reported in the literature (table 7). The sterility found in 351 heifers in the UNIVERSITY OF CALIFORNIA herd was 5.7 percent. This is only half that expected on the basis of gene frequency found in the sires. ALBRECHTSEN (1917) studied 22 Danish herds involving 2,209 heifers. For the individual herds, the sterility in heifers ranged from 0 to 9 percent, with a mean of 4.8 percent, or approxi-

mately one sterile female in 21. ECKLES (1929) summarized 29 years of breeding records collected at the UNIVERSITY OF MINNESOTA and branch stations. His data include 311 heifers two years of age. He found that 7.4 percent (or one in 13.5) did not conceive and were sold because of sterility.

Neither ALBRECHTSEN or ECKLES attempted a genetic analysis of their data. ALBRECHTSEN found that congenital stenosis of the cervical canal was relatively common and when present caused sterility. These sterile heifers became fertile after the cervical canal was enlarged by surgery. It is possible that congenital stenosis of the cervical canal is conditioned by heredity, perhaps by a single gene. ECKLES made no attempt to explain the sterility observed at the Minnesota station. "No attempt," he says, "is made to deal with the causes of the breeding difficultues or abortion, or with their treatment. To do so would lead into the fields of veterinary medicine, genetics, and nutrition, where the data concerning these fields are decidedly limited."

Throughout most of the period of ECKLES' study, the Minnesota herd was infected with Brucellosis, which caused much of the abortion and sterility in the older cows. Feeding and management conformed to the practices that prevailed in the higher producing herds and were therefore better than average. ECKLES definitely states that genetics is a factor in breeding difficulties and that Brucella infection caused many of the abortions and much of the subsequent sterility. Apparently, therefore, the failure of heifers to conceive was what led ECKLES to assume that heredity was important. According to the data of ALBRECHTSEN, ECKLES, and the California station, sterility in dairy heifers is common. Our data indicate that most of it, if not all, is caused by heredity. Possibly, environmental factors may be responsible for the failure of some heifers to conceive. Such causes, however, are probably rare under a good regime of feeding and management, when there is no infectious disease. Presumably, therefore, most of the sterility in heifers observed by ALBRECHTSEN and ECKLES was caused by heredity.

If there are two independent autosomal sex-limited genes, each of which alone conditions sterility, the proportion of sterility should be 0.115, or one heifer in nine. This is a much greater incidence than was found in the California herd and higher than the average found by ALBRECHTSEN, but only slightly higher than the maximum of 9 percent found in one herd. It is also slightly higher than the average of 7.4 percent reported by ECKLES for heifers at the Minnesota station. The discrepancy between the estimated sterility based upon the gene frequency found in the sires studied and the sterility observed in the California herd and that observed by ALBRECHTSEN and ECKLES may be caused solely by chance in sampling. There is certainly good agreement in all three studies on the percentage of sterility in heifers. It is possible, therefore, that the frequency based upon the percentage of sterility in heifers may be a more reliable criterion than that based upon the limited number of tested sires.

LINKAGE RELATIONS

No matings were made specifically to determine the linkage relations of the

mutant genes. A limited amount of information, however, was obtained from a few of the matings. The following combinations can be studied: (1) achondroplasia (a) strabismus (b) and flexed pasterns (c); and (2) cataract (d) and female sterility of the Jerseys (e).

There is difficulty in classifying the three phenotypes in the first combination. Strabismus is not manifest in a newborn calf and does not develop until the animal is about a year old. Flexed pasterns and achondroplasia are evident at birth. Since achondroplasia is a lethal and also affects the appendages, achondroplastic calves cannot be classified as to strabismus and flexed pasterns. All the data on linkage, therefore, come from five sires from appropriate matings where genotypes were determined by progeny tests (table 8). No. 320A was the sire or grandsire of all the animals that could be used in the linkage-relations analysis. The gene arrangement of 320A can be determined from tables 3 and 4.

The limited data available, assembled in table 8, indicate that the genes conditioning achondroplasia, strabismus, and flexed pasterns are genetically independent.

Five heifers homozygous for either cataract or female sterility yielded information on the relations of the two conditioning genes. All were sired by 333C, who was also a grandsire in all cases except one, in which he was a great-grandsire. Table 8 shows the gene arrangements of the animals involved. All five heifers in question are from recombination gametes, and the two genes are assumed to be independent.

Sire 386A proved heterozygous for imperfect epithelium, flexed pasterns, and cataract. It was also known that imperfect epithelium and flexed pasterns came from his sire, and cataract from his dam. No specific information could be obtained, however, on the status of any of the genes in this combination. The linkage relations of proportionate dwarfism and the female sterility of the Holsteins could not be studied in any combination.

DISCUSSION

It should be remembered that the sires were all selected at random so far as recessive genes are concerned. Throughout this paper, Jerseys and Holsteins have been treated in the main as one population. If the number of sires tested from each breed had been large, the breeds would have been considered separately.

All the European breeds of cattle have somewhat similar, although not identical, origins; and substantially similar types of mating systems have been employed in their development. Presumably, therefore, studies with other breeds may parallel rather closely the results obtained here, even though the specific genes found need not be the same in all cases. WRIGHT (1931) has shown that a large population mating at random can support a very large number of unfixed genes.

There is at least one other recessive gene that conditions sterility in cattle. ERIKSSON (1943) reports, in Swedish cattle, a form of sterility that affects both

TABLE 8

Tests bearing on linkage relations.

TEST ANIMAL		SIRE		DAM		RELATIONSHIP	REMARKS
NO.	GENE ARRANGEMENT	NO.	GENE ARRANGEMENT	NO.	GENE ARRANGEMENT		
			Achondroplasia (a); strabismus (b); flexed pasterns (c).				
375B	$\frac{+ \; b \; c}{+ \; + \; +}$*	320A	$\frac{a \; b \; c}{+ \; + \; +}$	375	$\frac{+ \; + \; +}{+ \; + \; +}$	—	a became disassociated from b and c in gametogenesis
428A†	$\frac{+ \; b \; +}{+ \; + \; +}$	320A	$\frac{a \; b \; c}{+ \; + \; +}$	428	$\frac{a? \; b? \; c}{+ \; + \; +}$	Daughter of 320A	b became disassociated from a and c either in 428A or 428
428B	$\frac{? \; ? \; c}{? \; ? \; c}$	320A	$\frac{a \; b \; c}{+ \; + \; +}$	428	$\frac{a? \; b? \; c}{+ \; + \; +}$	Daughter of 320A	c became disassociated from a and b in 428B or 428
433A	$a \; \frac{+}{+} \; c$	320A	$\frac{a \; b \; c}{+ \; + \; +}$	433	$\frac{a? \; b? \; c}{+ \; + \; +}$	Daughter of 320A	b became disassociated from a and c in 433A or 433
403C	$\frac{a \; b \; ?}{+ \; + \; +}$	386A	$\frac{+ \; + \; c}{+ \; + \; +}$	403	$\frac{a \; b \; c?}{+ \; + \; +}$	Daughter of 320A	Parental combination of a and b in 403C and 403
			Cataract (d); female sterility of Jerseys (e)				
581	$\frac{d \quad ?}{d \quad ?}$	333C	$\frac{d \; e}{+ \; +}$	30	$\frac{d \; e?}{+ \; +}$	Daughter of 333C	Recombination between d and e in 581 or 30
702	$\frac{d \quad ?}{d \quad ?}$	333C	$\frac{d \; e}{+ \; +}$	572	$\frac{d \; e?}{+ \; +}$	Daughter of 333C	Recombination either in 702 or 572
780	$\frac{? \quad e}{? \quad e}$	333C	$\frac{d \; e}{+ \; +}$	569	$\frac{d? \; e}{+ \; +}$	Daughter of 333C	Recombination either in 780 or 569
803	$\frac{? \quad e}{? \quad e}$	333C	$\frac{d \; e}{+ \; +}$	761	$\frac{d? \; e}{+ \; +}$	Granddaughter of 333C	Recombination either in 803 or 761
807	$\frac{d \quad ?}{d \quad ?}$	333C	$\frac{d \; e}{+ \; +}$	572	$\frac{d \; e?}{+ \; +}$	Daughter of 333C	Recombination either in 807 or 572

* Based upon 23 test progeny.
† Homozygosity based upon 12 test progeny only.

males and females. This form is conditioned by a single recessive gene with incomplete penetrance. The gene studied by ERIKSSON was not present in the California stock. It is conceivable that other genes may be found that condition sterility.

All the lethal genes thus far reported in cattle markedly affect morphological expression and are easily recognized. There are probably many more of this type yet to be found. Results of the California studies suggest that there may be a number of recessive lethal genes that interfere with normal physiological processes without causing visible morphological changes. If there are such genes, they may account for an appreciable percentage of abortions, stillbirths, and deaths soon after birth. The isolation and identity of genes of this type will depend upon specialized techniques, which must be devised to aid in their detection.

WRIEDT (1930) has pointed out that many great sires have proved heterozygous for lethals of one type or another. This fact may or may not be significant. Conceivably, heterozygotes may be favored in one way or another in selection, either natural or artificial. They may have greater viability, superior conformation, or the ability to yield more milk or fat. EAST (1936) has suggested how such a scheme might operate. Whatever the relationship may be, future research will have to provide the answer. It is sufficient at this time to emphasize that these studies clearly indicate that any bull (or cow) selected at random has a good chance to be heterozygous for lethal or other deleterious recessive genes. As a rule, only the sires that are greatly admired or favored for one reason or another are kept in service long enough, and with the proper mating tests, to be proved for recessive genes. The chances of proving for deleterious recessives a less favored sire that is kept in service less than three years are slight indeed.

Because of the low fecundity and long life cycles, many animal husbandmen and some geneticists have felt that it is impractical to attempt, with cattle and other domestic animals, a comprehensive genetic study patterned somewhat after the work done with laboratory animals. Those who would discourage such investigations recognize the value of progeny testing and the proving of sires for ability to transmit milk and fat yields and other characters of economic importance such as carcass yield. The results of the California dairy cattle breeding experiment, however, do not justify such a viewpoint. Agricultural colleges and experiment stations must maintain flocks and herds for teaching and for different types of research. If the personnel in charge are sufficiently interested, much valuable genetic data can incidentally be collected.

The more complete the mass of general genetic information concerning a species, the more intelligently can principles of heredity be applied to the improvement of characters of major economic importance. If rather complete genetic knowledge is available, such as has been compiled for some species of laboratory animals, undesirable genes can be controlled more easily in a program of inbreeding. In addition, light may be shed upon other, more compli-

cated genetic problems. The concentration of genes that condition high yield depends upon selection and a certain amount of inbreeding. More detailed results obtained from inbreeding will be presented in later publications.

A general study of all genes encountered can be incorporated into almost any program of proving sires if adequate data are collected and if the program is projected over a long time, as it should be in agricultural experiment stations. The use of artificial insemination on a large scale should facilitate the accumulation of all kinds of genetic data. The major requirements for such a program are interest and the keeping of complete records.

The authors are convinced that a general genetic program, as discussed, would be an asset to any specialized plan for improving an economic character such as milk and fat yields. Only individual experiment stations whose research personnel hold this view will attempt such a program. If breeding projects of this type were organized throughout most of the experiment stations, the mass of genetic information could be greatly augmented in a relatively short time. Eventually it should be possible to determine gene frequency, mutation rates, equilibrium points, selection values, and related problems, according to the techniques developed by FISHER (1930), HALDANE (1932) and the many papers of Wright, especially those of 1929 and 1937.

SUMMARY

The background of the UNIVERSITY OF CALIFORNIA breeding experiment with dairy cattle is presented, with a description of the original stock. Six sires, five Jersey and one Holstein, were selected at random so far as recessive genes were concerned and were tested for recessives by sire-daughter matings and other mating tests. Although some of the recessive genes carried by the sires may perhaps have escaped detection, there is no doubt concerning the eight that were positively identified.

Two lethal genes were found—one conditioning imperfect epithelium, the other an achondroplasia-like anomaly. The nonlethal genes were those conditioning proportionate dwarfism, flexed pasterns, strabismus, and congenital cataract. Two different forms of female sterility that prevented heifers from conceiving or producing offspring were also encountered. In the sterility found, only in animals of the Jersey herd did all the affected heifers manifest normal heat periods. The sterility found only in the Holstein breed was associated with an abnormal œstrous cycle, usually complete absence of heat. Both forms of sterility are sex-limited. There was no evidence that the fertility of heterozygous cows is impaired. One Jersey bull that was fully fertile was assumed to be homozygous for the Jersey type of female sterility. None of the sires proved were homozygous for the Holstein type of female sterility; but it is assumed that homozygous sires are fertile. The expected proportions of sterility in heifers based upon the gene frequencies found are compared with the actual figures recorded in the literature.

The matings provided some data concerning the linkage relations of certain combinations of the mutant genes. The matings involving the combination of

achondroplasia, strabismus, and flexed pasterns indicated that these genes are independent. Other matings indicated that cataract and the female sterility found in Jerseys are independent.

The feasibility of an organized program for amassing all kinds of genetic data on domestic animals is discussed.

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GENIC AND NONGENIC TRANSMISSION OF MUTATOR ACTIVITY

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INTRODUCTION

THE present study is based on the analysis of the recessive mutator gene (MAMPELL 1945). This mutator is located to the right of cinnabar in the second chromosome of *Drosophila pseudoobscura*, race B (*D. persimilis* Dobzhansky and Epling). When males are homozygous for this gene, mutations (usually belonging to the "Minute" phenotype) are induced in those males as well as in their offspring, no matter whether their offspring are male or female, homozygous or heterozygous. When females are homozygous for this gene, no such effects are observed. It has been assumed that homozygous mutator males produce a substance which induces the mutations; this substance would be transmitted to all the offspring through the cytoplasm of the sperm. The heterozygous males or homozygous wild type males would produce the wild type counterpart to the substance (insuring the stability of the genes) which would be transmitted in the same manner as the mutator substance.

MUTABILITY IN HOMOZYGOUS FEMALES

When a mutator male is outcrossed, an abundance of Minute mosaics appear in the heterozygous offspring. The mosaics are the result of mutation in the zygote and represent the residue of the mutation-inducing capacity of the father.

Homozygous mutator females do not produce mosaics in their offspring when outcrossed. Can they not transmit the mutator substance through the egg, even though they make it? Or do they not make the mutator substance?

When a mutation is induced in the tissue of a homozygote, one can speak of it as a primary mutation. When the mutation is induced in the tissue of a homozygote's heterozygous offspring (because of the mutator substance transmitted through the cytoplasm of the sperm), one may speak of it as a secondary mutation. If the mutations result in mosaics, in the former case they would be primary mosaics, in the latter case, secondary mosaics.

When homozygous males have a heterozygous father, primary mosaics do not ordinarily occur, because the wild type substance introduced with the sperm prevents mutation for several cell generations, or because a sufficient amount of mutator substance does not appear for several cell generations. Therefore, large, easily detectable mutated areas do not occur. When homozygous males have a homozygous father, they receive the mutator substance

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through the sperm; therefore, nothing would prevent the appearance of primary mosaics.

To illustrate this, we can compare the percentages of mosacis when males are crossed to wild type females and when they are crossed to heterozygous mutator females. In the former case only secondary mosaics will occur; in the latter case half the offspring will be homozygous for the mutator and therefore will be able to furnish primary mosaics. In the former case the rate is 2.69 percent (281 mosaics in 10,431 flies), in the latter 3.74 percent (247 mosaics in 6,610 flies). The increase would be ascribed mostly to the appearance of primary mosaics.

When the relative percentages of male and female Minute mosaics are compared in outcrossing and in inbreeding respectively, the increase of mosaics on inbreeding should come in the males only, if the homozygous females are unable to produce mutations (resulting in primary mosaics).

In the F_1 of a male outcross the male mosaics represent 93.92 percent of the female mosaics (185 mosaics in 5,218 males and 203 mosaics in 5,367 females). It is not difficult to understand why there should be fewer male than female mosaics. The females can show Minutes in the X chromosomes. In the males these would be lethal, thus probably eliminating all mosaics for sex-linked Minutes in the males. Now, in cultures resulting from inbreeding, the male mosaics represent 117.57 per cent of the female mosaics (919 mosaics in 13,740 males and 817 mosaics in 14,351 females). This considerable increase is to be ascribed to the appearance of primary mosaics in the males, or to the inability of the females to produce primary mutations.

The mutator gene does not operate in females.

THE INFLUENCE OF THE Y CHROMOSOME

Why does the mutator gene not operate in females? One of the outstanding genetic differences between the sexes is the presence of a Y chromosome in the male and its absence in the female. If a Y chromosome is necessary for the mutator gene to function properly, XXY females may be able to produce the mutator substance. Flies which are heterozygous for a race B X chromosome carrying Pointed and a race A X chromosome carrying Short Scutellum have three long inversions in the X and give a large amount of secondary non-disjunction. In this way XXY females can be obtained without difficulty.

It is found that XXY females give a considerable number of Minute mosaics when outcrossed, even though it is only about one third the number obtained from male outcrosses. However, it is possible that the number will increase further when a female has two Y chromosomes. This has not yet been tested. At any rate, it is clear that homozygous mutator females, provided that they have at least one Y chromosome, produce the mutator substance and transmit it through the gamete, as do the males.

A second Y chromosome does seem to increase the mutability in males. When wild type males are crossed to *Ss/Pt/Y* females, the nondisjunctional males will be wild type and XY; the regular males will be Pointed, and half

of these will get a Y chromosome from the mother and one from the father and therefore be XYY. If a second Y chromosome increases the mutability, there should be more mosaics among Pointed males than among wild type males due to greater frequency of primary mutation. The rate is 16.26 percent (27 mosaics in 166 flies) for the Pointed males and 11.39 percent (18 mosaics in 158 flies) for wild type males. Even though the numbers are not very significant, they indicate the possible existence of such an effect.

QUANTITATIVE AND QUALITATIVE VARIATIONS

Different mutation rates have been observed and can be explained on the basis of a susceptibility of the genes to the mutator. There are methods available for testing this assumption.

Mutator males were outcrossed to two kinds of females, those which had both chromosomes III and both chromosomes IV derived from a wild type strain which had never been selected for high mutability, and those in which one chromosome III and one chromosome IV was derived from the mutator strain constantly selected for high mutability; in both kinds of females the X and second chromosomes were derived from the wild type strain. In the former case the mutability as measured by Minute mosaics was 2.14 percent (161 mosaics in 6,597 flies), in the latter case it was 3.05 percent (139 mosaics in 4,557 flies). This indicates that the level of mutability is in part due to the relative number of genes susceptible to the mutator. Loci more or less mutable in respect to the activity of this mutator can be selected.

The variation in mutability, here tested, was quantitative. Automatically, different susceptibilities would have to express themselves qualitatively; this has also been observed, and accounts for the mutation fashions—that is, different frequencies of Minute, or Notch, or Delta or other phenotype mutations. Here, instead of selecting for general mutation frequency, specific regions would be selected for susceptibility, leading to repeated mutations at specific loci.

These data can also be used to confirm again that the mutations occur in the zygote, since the genes contributed by the mother can mutate only then. Furthermore, the data serve as additional evidence for a cytoplasmic factor. For, if the susceptible gene is contributed by the mother, the nonsusceptible gene and something which induces the mutation by the father, then that which makes the susceptible gene mutate is probably cytoplasmic.

Where the mutator substance is introduced with the cytoplasm of the sperm, it is clear that polyspermy may account for an additional high degree of variability.

THE EFFECTS OF FEMALE CYTOPLASM

When mutator males are crossed to heterozygous females, 3.74 percent mosaics are produced in the offspring as has been indicated in a previous paragraph. When males obtained from this cross are mated to their sisters, the percentage of mosaics in their progeny should be reduced to approximately one half, because here, only half the males are homozygous for the mutator.

Half the females to which these males are mated are heterozygous, half are homozygous; therefore, primary mosaics should occur more frequently among their offspring than in the previous generation. In view of this, an expectation of half the percentage seems conservative. The actual percentage is 0.96 (19 mosaics in 1,971 flies), which is only about one fourth. There is one essential difference between the mother of the male used in the first cross and of that used in the second cross. The former had received mutator substance since she had a homozygous father; the latter had not, since she had a heterozygous father. This indicates that the mutator gene, or the mutator particles produced by it, are more effective when in cytoplasm (contributed by the female) which has previously been influenced by mutator particles. This autocatalytic action is reminiscent of the Killer-kappa relationship in Paramecium (SONNEBORN 1943).

THE NATURE OF THE MUTATIONS

The kind of mutation produced by the mutator suggests deficiencies. Some evidence in favor of deficiencies has been presented in a previous paper (MAMPELL 1945).

A new indication that we are dealing with at least genetic deficiencies comes from a female that was heterozygous for several recessives, scarlet among others. This female was a Minute mosaic, and the side showing the Minute revealed also the scarlet eye.

It is easier to conceive of the mutator substance as inactivating the genes rather than physically removing them. One can only speculate about the role of the heterochromatic Y chromosome in the mutator substance. One may assume that the mutator-plus gene produces a wild type substance, connected with heterochromatin, which is concerned with the normal reproduction of all genes; the mutator substance may consist of a qualitatively different heterochromatin, and by incorporating that into the chromosomes here and there when taking part in their reproduction may induce the mutations. Strange heterochromatin does seem to interfere with the normal functioning of genes as we can see in the numerous cases of variegation. There, mutation may also be the cause of the disturbance, at least in some cases. Here, mutation probably means a permanent complete inactivation of certain genes, thereby suggesting deficiencies, because of heterochromatin interference with the normal functioning of those genes. (For full discussion of problems bearing on this point see MIRSKY 1943; WRIGHT, 1945.)

Heterochromatin is, of course, not a chemically defined substance. The heterochromatic regions in the chromosomes are evidently concerned with the formation of both ribose and desoxyribose nucleic acid. One should not exclude the possibility that the formation of one would be primarily located in Y and autosome-heterochromatin, and of the other in X heterochromatin. The numerical relationship in the mutability of mutator males and females with varying amount of Y heterochromatin would almost point toward such an interpretation.

AN INFECTED D. PSEUDOOBSCURA STRAIN

Soon after work with the mutator strain was started, other strains of *D. pseudoobscura* seemed to show an increased mutability of the same general nature as existed in the mutator strain. Evidently the degree of mutability could be increased or decreased by selection. There is, of course, always a suspicion of genetic contamination, but the work with these strains shows that the mutator gene as such is evidently not involved. The over-all mutability is here much lower and, in spite of constant selection through many generations, it could so far not be increased beyond 0.42 percent. Also, the out-

TABLE 1

HB outcrosses.

	♂ OUTCROSS					♀ OUTCROSS				
	+Rp ♀♀ × +HB ♂♂					+HB ♀♀ × +Rp ♂♂				
	from cultures with 0.00% mos. (0 in 15,643)		from cultures with 0.41% mos. (44 in 10,405)			from cultures with 0.41% mos. (44 in 10,405)		from cultures with 0.00% mos. (0 in 15,643)		
	% MOS.	% M	MOS.	M	NO. FLIES	% MOS.	% M	MOS.	M	NO. FLIES
F_1	0.18	0.13	8	6	4,528	0.02	0.13	1	8	6,180
F_2	0.01	0.03	1	2	6,913	0.08	0.06	13	10	15,978
F_3	0.19	0.01	14	1	7,215	0.07	0.06	11	9	14,967

crosses to a nonmutating strain give different results from what would be expected if the mutator gene were present.

The "Hope, British Columbia" (HB) strain of *D. pseudoobscura*, race B, has continuously been selected for high mutability. The same system of outcrosses to the "Reedsport" (Rp) strain was then practiced as with the mutator strain (MAMPELL 1945). The results are given in table 1.

Comparing the results with those obtained by outcrossing the mutator strain, it is seen that the F_3 of the male outcross gives a mutability about as great as the F_1. This is unexpected. In the female outcross the F_2 gives about as great a mutability as the F_3. Again this is unexpected. Neither an autosomal nor a sex-linked dominant or recessive gene can well account for these results.

Without attempting to explain these observations here, we are obviously dealing with a different phenomenon. How did this strain get its mutability? As a possibility, it was assumed that the mutator substance might have a virus nature and that other strains may become infected with it. This seemed like a serious possibility, because the writer had observed what seemed like an increased mutability in various strains of different species of Drosophila which were kept in his laboratory. The experiments which were designed to test this hypothesis of infection will be described in the next section.

In the HB strain the male mosaics are about twice as frequent as the female mosaics. This probably means that only secondary mosaics appear in females, whereas the males get the benefit of primary mutation as well. Since the mutator gene is presumably not present in this strain, one may assume that mutator particles are self-reproducing in males but not in females. They would need a Y chromosome for their reproduction. This would explain why the F_3 of the male outcross shows more mutations than the F_3 of the female outcross. The female would draw on the supply of mutator particles which come from the parents and, without a Y chromosome, would not replenish the supply. It is now known that the mutator gene can function only in the presence of a Y chromosome. Indeed, one may say that the Y chromosome is the primary agent of this mutability; and the mutator gene would merely enhance the effect which potentially exists in normal flies.

There is still, aside from minor discrepancies, the serious discrepancy of the F_2 of the male outcross. Here, as in a similar case reported above, one may give the possible interpretation that the mutator particles can become functional only in cytoplasm, contributed by the female, which has previously been under the influence of mutator particles. Whereas they would continue to be replenished by continuous passage through males, they would not be turned into functional mutator particles in the F_1 males, because the mother's cytoplasm was not properly impressed by mutator particles; but the mother of the F_2 male would have the proper cytoplasm. Therefore, those males would give mosaics among their offspring.

This may not be the most satisfactory explanation; but at this time, without a careful study of the problem, it would not be profitable to speculate further.

INFECTION EXPERIMENTS

In order to test for possible virus transmission, pairs of *D. melanogaster* were placed into *D. pseudoobscura* cultures containing an average of 60 adult flies. From the *D. melanogaster* offspring thus reared in cultures with *D. pseudoobscura* adults, pairs were selected at random and raised individually; the percentage of all mosaics and dominant mutations in their offspring was used as an index of the mutability.

Where one is dealing with low rates of visible mutations, one must take care to exclude the personal factor. Visible mutations are likely to be found where they are expected, and to be overlooked where they are not expected. The method here employed insures the elimination of such prejudice, since the writer did not know in any case whether a culture belonged to the experimental or the control series, since all cultures carried merely a number, and no designation referring to the kind of *pseudoobscura* culture in which the grandparent had been placed. It is the opinion of the writer that such precautions are necessary.

When *melanogaster* flies are raised in cultures with mutator males, their descendants show a mutability of 0.05 percent (22 mutations in 44,919 flies); when they are raised in cultures with mutator females, their descendants show

a mutability of 0.01 percent (2 mutations in 15,013 flies). However, when *melanogaster* flies are raised in cultures with wild type males (which make no mutator substance) their descendants also produce a mutability of 0.05 percent (16 mutations in 31,774 flies); those raised in cultures with wild type females show a mutability of 0.01 percent (one mutation in 12,916 flies). A mutability of 0.01 percent is normal for the Canton-S strain used in this experiment (two mutations in 17,588 flies). It seems that the primary effect is not so much due to the mutator gene but to the Y chromosome. It is obviously concerned with mutability, as has been demonstrated. In that case, when the *melanogaster* flies are raised in cultures with females which have a Y chromosome, mutations should be induced; mutability obtained from descendants of *melanogaster* raised in cultures with XXY females again seems to be increased (0.03 percent, five mutations in 14,746 flies). Summarizing, the descendants of *melanogaster* raised in cultures with *pseudoobscura* males give a mutability of 0.05 percent (38 mutations in 76,693 flies); those raised in cultures with *pseudoobscura* females give a mutability of 0.01 percent (three mutations in 27,929 flies). Chi square test shows this difference to have a significance at the one percent level. The influence of the concentration of infecting flies on the degree of mutability is now being tested. An interpretation of this possible "long distance" effect of the Y chromosome on mutability will be given later.

PERSISTENCE OF MUTABILITY AFTER INFECTION

When strains which have received a mutability by infection are continued, the mutability tends to persist. The "high" strain of *melanogaster*, derived from, but not including the originally infected cultures, has a mutability of 0.07 percent (20 mutations in 28,433 flies). The normal untreated "low" strain has a mutability of 0.01 percent (two mutations in 17,588 flies).

It is the continuation of the mutability in the infected strain which tends to support the virus hypothesis; for the mutator particles seem to reproduce in the *melanogaster* strain. This would demonstrate the relative independence of the mutator particles of any genes specifically concerned with their maintenance. The proper environment exists for the perpetuation of the "symbiotic" as well as the "parasitic" particles.

CONCLUSION

The mutator gene, provided a Y chromosome is present, is responsible for the production of a mutator substance which is responsible for inducing mutations and which is transmitted through the cytoplasm of egg and sperm. This mutator substance consists of mutator particles, possibly single molecules, important in gene reproduction and self-reproducing to some extent. It seems that the wild type counterpart, the "mutator-plus" (mu+) particles behave in a similar fashion.

Since the Y chromosome is connected with the source of essential substance on which the genes depend, it may be questioned how the females would get along without a Y chromosome; but it has been shown that the mutator sub-

stance is transmitted to all offspring through the sperm, and the same has been assumed for the mutator-plus substance. Since every female has had a father, she has received this material and would be under the influence of this paternal effect.

The action of the mutator particles may be to incorporate a kind of substance into the genes, which is unnatural as far as the genes are concerned. This may lead to their inactivation or loss of normal function. Once the new configuration has come about, the gene would reproduce itself in this way and the mutation would be stable. The mutator-plus particles may incorporate into the genes a more malleable substance which each gene would use to insure continuation of its own specificity.

It seems that the mutator particles dissociate themselves from the organism where they were produced and are transferred to other organisms, and, like a virus, they reproduce themselves there. If the assumption of genes mutating in strange heterochromatin is correct, then any strange heterochromatin may produce this effect. It would then be clear why *melanogaster* do not only mutate after the passive transfer of *pseudoobscura* mutator particles, but after the transfer of *pseudoobscura* mutator-plus particles as well.

In this way, a perfectly normal constituent of an organism, produced by that organism, could become a virus when transferred to another organism. Thus one can originate a new virus. (For further discussion see DARLI GTON 1944.)

One may use the mutator phenomenon as an example of the close relationship between gene, cytoplasmic component, and virus.

SUMMARY

The genetic information concerning the mutator gene has been extended. Females are unable to induce primary or secondary mutations, because they do not produce the mutator substance.

When they are given a Y chromosome, they can make and transmit the mutator substance, thus inducing primary and secondary mutations. Primary mutation is evidently increased in the male by a second Y chromosome.

Quantitative and qualitative variations in the mutability can be shown to be due to differential susceptibility of the genes to the mutator.

When the mutator is in cytoplasm (from the female) which has previously been influenced by the mutator substance, its effectiveness may be increased.

The mutations are genetic deficiencies, conceivably representing heterochromatin inactivations of genes rather than physical deficiencies.

High mutability of the same appearance as in the mutator strain has been observed in other strains; there, it is not due to the mutator gene. The possibility of transmission of the mutability by infection was tested.

Pairs of *melanogaster* were placed in cultures with adult *pseudoobscura* flies. Mutability is increased in the descendants of *melanogaster* raised in cultures with mutator or wild type males; it is not increased in cultures with mutator or wild type females.

The mutability in infected strains tends thereafter to persist.

A hypothesis as to the nature and mode of action of the mutator particles and the mutator-plus particles has been presented. It has been argued that perhaps the normal symbiotic particles of one species may become parasitic virus particles when transferred to another species.

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THE PHENOTYPES OF HOMOZYGOTES AND HEMIZYGOTES OF POSITION ALLELES AND OF HETEROZYGOTES BETWEEN ALLELES IN NORMAL AND TRANSLOCATED POSITIONS[1]

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EXCEPT for rare cases of additive expression, it is a rule which is nearly without exception that the heterozygote between two alleles is phenotypically either identical with one of the two homozygotes or intermediate between them. In experiments involving position effects of the fourth chromosome normal allele of cubitus interruptus (*ci*) of *Drosophila melanogaster*, several cases have been found where this rule does not hold but where the heterozygotes lie phenotypically outside of the range delimited by the homozygotes.

MATERIAL AND METHODS

DUBININ and SIDOROW (1934) showed that the dominance of the normal, +allele over its recessive allele *ci* is frequently weakened when a translocation occurs near the *ci* locus. To gain additional data on the nature of this effect, males of an isogenic wild type stock (Canton-S) were irradiated with a 4000r dose of X-rays and mated to females homozygous for *ci ey*^{R}. The offspring were all heterozygous +/*ci ey*^{R} and most of them were normal phenotypically (*ey*^{R}=allele of eyeless recessive chromosome 4). A small number, however, showed various degrees of interruption of the fourth vein. Nineteen such individuals and the stocks derived from them were analyzed (STERN, SCHAEFFER, and HEIDENTHAL 1946). All proved to contain "position alleles," *R*(+), defined as rearrangements ("*R*") involving the neighborhood of the + allele at the *ci* locus.

Crosses were made between *ci ey*^{R}/*ci ey*^{R} females and *F* (+)/*ci ey*^{R} males of all 19 different *R*(+) stocks. The offspring of these crosses consisted of round eyed *R*(+)/*ci ey*^{R} and small eyed *ci ey*^{R}/*ci ey*^{R} sibs, disregarding some additional individuals due to unbalanced genotypes and to nondisjunction. The flies were classified as to degree of interruption of veins by arbitrarily distinguishing five classes—0, 1, 2, 3, 4—from no interruption to complete absence of the vein. (This designation of the five phenotypic classes replaces the designation N, 0, 1, 2, 3 prior to 1946.) An index of expressivity was then calculated by giving each class the weight of its class designation 0–4 and determining the mean of the distribution. Separate means for each culture were obtained for females and males of each of the two genotypes *R*(+)/*ci ey*^{R} and *ci ey*^{R}/*ci ey*^{R}, and total mean values for each sex and genotype were deter-

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mined from the sum of the distributions of all cultures of each $R(+)$ allele. These values, for the females, lie between 1.03 and 3.44 for $R(+)/ci\ ey^R$ and between 2.00 and 2.84 for $ci\ ey^R/ci\ ey^R$; for the males, between 0.96 and 3.80 for $R(+)/ci\ ey^R$ and between 2.34 and 2.97 for $ci\ ey^R/ci\ ey^R$ (see STERN, SCHAEFFER, and HEIDENTHAL 1946, table 1).

The different degrees of expressivity are the result both of intrinsic differences between the different $R(+)$ alleles and of environmental differences influencing the degree of vein interruption. Since environmental variations

TABLE 1

Distribution of differences between the means of expressivity of ci ey^R flies and the means of nineteen different R(+)/ci ey^R heterozygotes. In all cases except R^{15}(+) (see special treatment) differences are based on comparisons of sibs.

DIFFERENCES BETWEEN MEANS OF *ci ey^R/ci ey^R* AND *R(+)/ci ey^R*.							
		>1.50	1.00–1.49	0.50–0.99	0.0–0.49	−(0.01–0.49)	−(0.50–0.99)
Stock number of $R(+)$ allele	♀♀	11	19, 20	1, 5, 6, 8, 10, 14	16, 18, 21	7, 9, 12, 13	2, 3, 15
Total	19	1	2	6	3	4	3
Stock number of $R(+)$ allele	♂♂	11	1, 5, 6, 10, 14, 20	8, 16, 18, 19	7, 9, 13, 21	12, 15	2, 3
Total	19	1	6	4	4	2	2

should affect $R(+)/ci\ ey^R$ and $ci\ ey^R/ci\ ey^R$ flies similarly, the influence of environment can be largely excluded if the $R(+)$ alleles are not characterized simply by their values of expressivity but rather by the differences between the expressivity values of the $ci\ ey^R/ci\ ey^R$ individuals and their like-sexed $R(+)/ci\ ey^R$ sibs. These difference values are given in table 1. A positive difference means that the heterozygote is less abnormal than the $ci\ ey^R/ci\ ey^R$ homozygote, while a negative difference signifies the opposite. It is seen that seven $R(+)$ alleles give negative differences for females and four for males. The $R(+)$ alleles giving negative differences for males are included among those giving negative female differences. In other words, four $R+$ alleles, numbered $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ in chronological order of their initial discovery, produce in both sexes, as heterozygotes, more extreme interruption than $ci\ ey^R/ci\ ey^R$ sibs, while $R^7(+)$, $R^9(+)$, and $R^{13}(+)$ are more extreme than $ci\ ey^R/ci\ ey^R$ in females only, but less extreme in males. The remaining 12 $R(+)$ alleles give positive differences for both sexes.

$R^7(+)$, $R^9(+)$, and $R^{12}(+)$ heterozygotes are only slightly different from the $ci\ ey^R/ci\ ey^R$ homozygotes so that the fact of positive differences for the males and negative ones for the females may represent only chance deviations from a true value close to zero. Statistically the negative differences for $R^9(+)$ and $R^{13}(+)$ females are not significantly different from zero.

In the following an analysis of the position-alleles $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$ is presented. Of these, $R^3(+)$, which is a more complicated rearrangement than the others, will be treated last.

CYTOLOGICAL DETERMINATIONS

$R^2(+)$. Translocation between the right arm of chromosome 3("3R"), broken at the very end of section 82D (BRIDGES map, 1935) and chromosome 4, left arm. The break in 4L is not recognizable in salivary gland nuclei.

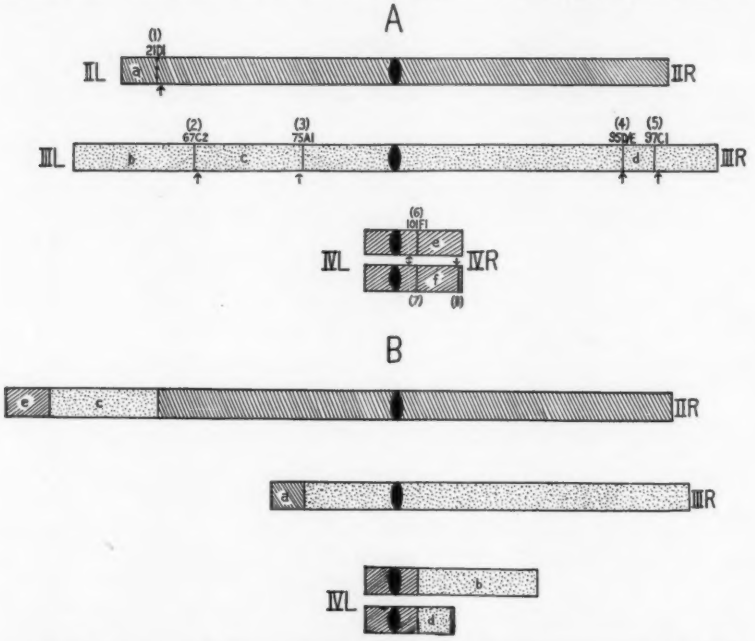

FIGURE 1.—Diagram of the $R^3(+)$ rearrangement.—A. Normal chromosomes 2, 3, and 4 (the latter split into two chromatids). Position of breakage points are indicated.—B. The rearranged chromosome elements.

Oogonial metaphases show a rod shaped chromosome considered to be 3L and a very unequal armed chromosome considered to be 3R and 4R.

$R^{12}(+)$. Translocation between 3R and 4R. Break in 3R immediately following 98F1; in 4R in region 101 very close to the kinetochore. Oogonial metaphases of flies heterozygous for the translocation appear nearly normal with one of the two spherical chromosomes slightly smaller and more diffusely stained than the other. Presumably the diffuse element corresponds to the base of chromosome 4 and the tip of 3R attached to it.

$R^{15}(+)$. Insertion of a section of 4R into 2L. Breaks in 4 immediately before 101F1 and immediately after 102C4. Inserted in 30A between the first and last

heavy band—that is, inside the capsule. Oogonial metaphases of flies heterozygous for the translocation appear normal except for one of the two spherical chromosomes slightly smaller than the other. The smaller chromosome is the deficient one.

$R^{3}(+)$. A complex rearrangement, involving at least six breaks (see fig. 1A). (1) in 2L to the right of 21D1; (2) and (3) in 3L to the right of 67C2 and to the left of 75A1 respectively; (4) and (5) in 3R to the left of 95D/E and of 97C2 respectively; and (6) in 4R to the left of 101F1, very close to the kinetochore. In the rearrangement the nearly complete right arm of 4 has become translocated at the 67C2 end to the segment 67C2–75A1 of 3L, which in turn has become attached with its 75A1 end to the 21D1 breakage point of 2L. The whole rearranged sequence is shown in fig. 1B.

It is seen that the short section 95D/E-97C1 of 3R is not a part of the rearranged sequence. This section occurs as an independent chromosome unit (KODANI and STERN 1946). In salivary gland nuclei its 95D/E end is regularly embedded in the chromocenter. Frequently the 97C1 end is also joined to the chromocenter so that the section 95D/E-97C1 forms a short loop. Presumably the proximal end of the fragment is translocated to a base of chromosome 4 which thus would be present in addition to the other base of 4 which carries the translocated tip of 3L. It is possible that the two bases of 4 originated as a result of breaks induced in the two chromatids of chromosome 4 transmitted by the irradiated sperm. It is not clear how the 97C1 end of the excised section terminates. Its tendency to synapse with the chromocenter suggests the possibility that the section became inserted in a chromatid of 4 which itself became deleted of nearly all of 4R excepting the base and very tip.

Oogonial metaphases of flies heterozygous for the rearrangement possess normal X chromosomes, one normal chromosome each of 2, 3, and 4, and in addition a chromosome whose one arm is longer than a normal arm, another chromosome whose one arm is shorter than a normal arm, and a short rod-shaped chromosome two to three times longer than a normal chromosome 4. Obviously the unusually long arm represents the main part of 2L with parts of 3L and 4R added and the unusually short arm the base of 3L with the short tip of 2L attached to it. The rod shaped chromosome should be either made up of the base of 4 and the translocated tip of 3L, or of the base of 4 and the translocated excision from 3R. Either interpretation involves the assumption that one of the two elements, be it base of 4 and 3R fragment, or base of 4 and 3L tip are too small to be visible in oogonial preparations. Considering the relative length of the 3R and 3L fragments in the salivary chromosomes, it would be expected that the short section 95D/E-97C1 of 3R would be the one invisible. This was proven by simultaneous analysis of ganglionic metaphase chromosomes and of salivary gland chromosomes of certain larvae (KODANI and STERN 1946).

HEMIZYGOTES

It had been found by DUBININ and SIDOROW (1934) that the four different $R(+)$ alleles which they investigated in hemizygous condition led in this state

to normal, not interrupted vein phenotypes. This seemed surprising in view of the fact that the same $R(+)$ alleles in heterozygous combination with *ci* behaved like mutant *ci* alleles themselves.

Similar results were obtained with the four $R(+)$ alleles of the present study (table 2). Instead of using haplo-4 flies as hemizygotes, as the Russian workers did, the hemizygotes of $R^{2}(+)$, $R^{3}(+)$, $R^{12}(+)$, and $R^{15}(+)$ contained the Minute-4 (*M-4*) carrying chromosome 4 which is deficient for a region including the *ci* locus. Most of the hemizygotes were obtained in the offspring of crosses of $R(+)/ci\ ey^{R}$ *by* $M\text{-}4/+$ parents. The resulting *M-4* flies were of the con-

TABLE 2

Hemizygotes for R(+) alleles.

GENOTYPE	TEMP.	PHENOTYPIC CLASSES			
		♀♀		♂♂	
		0	1	0	1
$R^{2}(+)/M\text{-}4$	14°–16°	65	—	76	—
$R^{3}(+)/M\text{-}4$	14°–16°	21	2	27	1
	26°	31	—	27	—
$R^{12}(+)/M\text{-}4$	16°	13	—	3	—
	26°	2	—	5	1
$R^{15}(+)/M\text{-}4$	15°–16°	62	2	51	8
	26°	22	1	20	—

stitutions $M\text{-}4/ci\ ey^{R}$ and $M\text{-}4/R(+)$. The former are known to possess wing interruptions nearly exclusively of the extreme classes 3 and 4. The presence of normal (class 0) or nearly normal (class 1) individuals was taken as proof of the presence of $M\text{-}4/R(+)$ individuals. There was a possibility that some of the Minute flies classified as $M\text{-}4/R^{15}(+)$ were in reality $Df4[R^{15}(+)]/+$, where $Df4[R^{15}(+)]$ signifies the deficient chromosome 4 of $R^{15}(+)$ and $+$ the normal chromosome 4 of the $M\text{-}4/+$ parent. A similar possibility existed in case of the $R^{3}(+)$ experiments where haplo-4 individuals might have originated in consequence of nondisjunction, thus obtaining no chromosome 4 from the $R^{3}(+)$ parent and a chromosome 4 with the normal allele from the $M\text{-}4/+$ parent. In order to exclude these sources of error which were present in the tests carried out at 15°–16°, the experiment was modified by using $M\text{-}4/ey^{D}$ instead of $M\text{-}4/+$ parents and inspecting the *M-4*, not ey^{D} offspring only (ey^{D} = eyeless-dominant, chromosome 4). This latter method or similar ones were adopted for all experiments carried out at 26°C. Their outcome did not differ essentially from that of the earlier experiments.

At the usual culture temperatures of 25°–26° the hemizygotes, with two exceptions only, were phenotypically normal notwithstanding the fact that the $R(+)$ alleles tested are unusually effective in producing extreme *ci* phenotypes in heterozygotes with *ci*. At lower temperatures, where the degree of interrup-

tion, if any, is known to be shifted toward greater expressivity, several $M\text{-}4/R^{3}(+)$ and $M\text{-}4/R^{15}(+)$ individuals of vein abnormality class 1 appear. While it is difficult to be certain that the frequency of these abnormal $M\text{-}4/R(+)$ individuals is greater than expected in nonposition allele $M\text{-}4/+$ flies, comparisons with earlier data on $M\text{-}4/+$ (STERN and SCHAEFFER 1943b) suggest that this is the case. This would indicate that the position-alleles $R^{3}(+)$ and $R^{15}(+)$ are slightly less effective in producing a normal phenotype than the + allele in its unchanged locus.

HOMOZYGOTES

Of the four rearrangements, $R^{3}(+)/R^{3}(+)$ and $R^{12}(+)/R^{12}(+)$ proved to be inviable. Homozygous $R^{2}(+)/R^{2}(+)$ and $R^{15}(+)/R^{15}(+)$ survive, and stocks

TABLE 3

$R^{2}(+)$ homozygotes.

♀♀					♂♂				
0	1	2	3	4	0	1	2	3	4
4	1	58	33	—	1	1	45	35	—

TABLE 4

$R^{2}(+)$ homozygotes and ci ey^{R}/ci ey^{R} raised in the same cultures.

$R^{2}(+)/R^{2}(+)$										$ci\ ey^{R}/ci\ ey^{R}$									
♀♀					♂♂					♀♀					♂♂				
0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
—	8	29	8	—	—	4	21	12	—	—	42	222	83	—	—	19	178	333	19

were established which were proved to be homozygous both genetically and cytologically.

In conformity with the observations of DUBININ and SIDOROW on three of their $R(+)$ alleles in homozygous condition, $R^{15}(+)/R^{15}(+)$ proved to be completely normal (203 ♀♀, 184 ♂♂).

Homozygotes for $R^{2}(+)$ are not normal but show interruptions of venation very similar in expressivity to those of homozygous mutant *ci/ci* (table 3). In order to compare the expressivity of $R^{2}(+)/R^{2}(+)$ and *ci/ci* under as similar conditions as possible, females of the former genotype inseminated by males of the same constitution and *ci* ey^{R}/*ci* ey^{R} females inseminated by *ci* ey^{R}/*ci* ey^{R} males were placed together in the same culture bottles and their offspring classified for degree of interruption of veins (table 4). It is seen that $R^{2}(+)$ homozygotes are slightly less extreme than *ci* ey^{R} homozygotes. The difference in expressivity is significant for the males only.

The behavior of $R^{2}(+)$ homozygotes shows that the findings of DUBININ

and SIDOROW as to normality of homozygotes of $R(+)$ position alleles at the *ci* locus cannot be generalized. Of the total of five types of homozygotes described by DUBININ and SIDOROW and by the present authors, four exhibit the normal and one a mutant phenotype.

HETEROZYGOTES

The heterozygotes between any one of the four $R(+)$ alleles and the normal allele in the Canton-Special stock, $+^{C}$, were phenotypically normal, as shown by the normal venation of all but one offspring from crosses of $R(+)/ci\ ey^{R} \times +^{C}/+^{C}$. Half of the flies must have been heterozygous for $R(+)$ and $+^{C}$. The numbers observed in the four types of crosses were: $R^{2}(+)$, 233 ♀♀, 233 ♂♂ (one of these showed venation class 1); $R^{3}(+)$, 145 ♀♀, 171 ♂♂; $R^{12}(+)$, 153 ♀♀, 141 ♂♂; $R^{15}(+)$, 203 ♀♀, 184 ♂♂.

More interesting are the heterozygotes between the $R(+)$ alleles and *ci* as obtained from crosses of $ci\ ey^{R}/ci\ ey^{R}$ ♀♀ × $R(+)/ci\ ey^{R}$ ♂♂. The data will be presented in detail in order to enable the reader to form his own judgment as to the interpretation of phenotypes. It will be noted that different cultures of the same genotypes sometimes show phenotypic distributions which are different beyond chance expectations. On the whole, however, the homogeneity of the data in the face of the delicate response of *ci* venation to various environmental and genetic modifiers is striking.

$R^{2}(+)/ci\ ey^{R}$

The expressivity of this genotype can be judged from five separate cultures (table 5). The mean values for the expressivity of the $R^{2}(+)/ci\ ey^{R}$ flies of both

TABLE 5

F_1 of five separate cultures of 2 ci ey^{R}/ci ey^{R} ♀♀ × 1 $R^{2}(+)$/ci ey^{R} ♂.

CULTURE NUMBER	$R^{2}(+)$										$ci\ ey^{R}$									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	—	7	50	7	—	—	2	50	11	2	8	31	15	—	—	7	22	28	—
2	—	—	10	38	6	—	—	1	52	14	—	10	25	18	2	—	—	22	40	2
3	—	—	14	39	3	—	—	2	60	13	1	10	21	15	1	—	5	23	36	1
4	—	—	3	40	8	—	—	2	38	10	—	3	23	13	4	—	1	9	36	4
5	—	4	13	32	1	—	—	4	37	3	2	2	23	12	—	—	—	20	15	3
Total	—	4	47	199	25	—	—	11	237	51	5	33	123	73	7	—	13	96	155	10

sexes and their $ci\ ey^{R}/ci\ ey^{R}$ sibs, calculated separately for each culture and for the total of all, are listed in table 6.

The differences in mean values between $R^{2}(+)/ci\ ey^{R}$ and $ci\ ey^{R}/ci\ ey^{R}$ for the females vary between −0.45 and −0.95 with an average difference of −0.71. For the males they vary between −0.30 and −0.77 with an average difference of −0.64. In every case the $R^{2}(+)/ci\ ey^{R}$ value is greater than the corresponding $ci\ ey^{R}/ci\ ey^{R}$ value, showing that the heterozygotes are more

TABLE 6

Means of expressivity of $R^2(+)$ heterozygotes and ci ey^R sibs listed in table 5.

CULTURE NUMBER	$R^2(+)$		*ci ey^R*		DIFF. BETWEEN MEANS	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	3.00	3.14	2.05	2.37	−0.95	−0.77
2	2.93	3.19	2.22	2.69	−0.71	−0.50
3	2.80	3.15	2.10	2.51	−0.70	−0.64
4	3.10	3.16	2.42	2.86	−0.68	−0.30
5	2.60	2.98	2.15	2.55	−0.45	−0.43
Total	2.89	3.13	2.18	2.59	−0.71	−0.64

extreme than the mutant homozygotes. Since the latter are themselves slightly more extreme than $R^2(+)/R^2(+)$, the $R^2(+)/ci\ ey^R$ heterozygotes are more abnormal than either homozygote.

$$R^{12}(+)/ci\ ey^R$$

The original data for four cultures and the mean values of expressivity are listed in tables 7 and 8. The values for $R^{12}(+)/ci\ ey^R$ females vary from 2.21

TABLE 7

F_1 of four separate cultures of 2 ci ey^R/ci ey^R ♀♀ × 1 $R^{12}(+)$/ci ey^R ♂.

CULTURE NUMBER	$R^{12}(+)$										*ci ey^R*									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	2	3	8	30	1	—	2	3	23	—	—	4	25	25	2	—	1	16	22	—
2	—	8	5	16	3	—	1	4	23	1	—	3	11	5	—	—	3	9	9	—
3	—	8	10	29	2	—	5	9	20	2	—	12	27	23	3	—	2	12	26	2
4	3	11	7	25	1	—	1	6	22	—	—	15	21	11	—	—	1	10	19	2
Total	5	30	30	100	7	—	9	22	88	3	—	34	84	61	5	—	7	47	76	4

TABLE 8

Means of expressivity of $R^{12}(+)$ heterozygotes and ci ey^R sibs listed in table 7.

CULTURE NUMBER	$R^{12}(+)$		*ci ey^R*		DIFF. BETWEEN MEANS	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	2.57	2.75	2.42	2.54	−0.15	−0.21
2	2.44	2.83	2.11	2.29	−0.33	−0.54
3	2.51	2.53	2.26	2.67	−0.25	+0.14
4	2.21	2.72	1.91	2.69	−0.30	−0.03
Total	2.43	2.70	2.20	2.57	−0.23	−0.13

to 2.57, with an average of 2.43, for *ci ey^R/ci ey^R* females from 1.91 to 2.42, with an average of 2.20. The male values vary from 2.53 to 2.83, average 2.70, and from 2.29 to 2.69, average, 2.57, respectively. In seven out of eight comparisons of $R^{12}(+)$ heterozygotes with *ci ey^R* homozygotes the mean expressivity of $R^{12}(+)$/*ci ey^R* is larger than that of *ci ey^R/ci ey^R* sibs. In the one comparison which gives an opposite result the difference is statistically not significant. The average difference for the females is −0.23, for the males −0.13. Both these values, though small, are significantly different from zero.

$R^{15}(+)$/*ci ey^R*

The cross of $R^{15}(+)$/*ci ey^R* × *ci ey^R/ci ey^R* results, among both sexes, in the following four different viable genotypes: (1) Chromosome 3 with $R^{15}(+)$

TABLE 9

F_1 of four separate cultures of 2 ci ey^R/ci ey^R ♀♀ × *1 $R^{15}(+)$/ci ey^R* ♂.

CULTURE NUMBER	$R^{15}(+)$										*ci ey^R*									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	—	2	17	1	—	—	3	11	—	—	5	14	1	—	—	8	23	10	—
2	—	—	1	23	5	—	—	2	26	—	2	16	20	8	—	—	5	27	20	—
3	—	—	2	15	—	—	—	4	12	—	—	9	11	10	—	—	6	17	14	1
4	—	—	—	28	7	—	—	1	30	2	—	8	23	21	1	—	9	28	32	2
Total	—	—	5	83	13	—	—	10	79	2	2	38	77	40	1	—	28	95	76	3

insertion/normal 3, deleted 4/normal 4; (2) 3 with $R^{15}(+)$ insertion/normal 3, normal 4/normal 4; (3) normal 3/normal 3, deleted 4/normal 4; (4) normal 3/normal 3, normal 4/normal 4. Flies of genotype (3) are deficient for a section of chromosome 4. They appear phenotypically as Minutes, with extreme expression of *ci* but not eyeless. Obviously the deleted section includes the *ci* locus but not that of *ey*. Genotype (1) $R^{15}(+)$/*ci ey^R* is phenotypically normal-eyed, genotype (2) insertion $R^{15}(+)$/*ci ey^R/ci ey^R* appears as eyeless, as does genotype (4) *ci ey^R/ci ey^R*. Provided that genotypes (1), (2) and (4) are equally viable, a ratio of 1 $R^{15}(+)$: 2 *ci ey^R* phenotypes is expected. This expectation is approached closely as shown in table 9 (the table does not include 55 Minute type flies of genotype 3). Due to the fact that in these cultures flies with *ci ey^R* phenotypes include the two different genotypes (2) and (4), the mean expressivity of *ci ey^R* among these flies cannot be used as a control for the expressivity of their $R^{15}(+)$/*ci ey^R* sibs in the same sense in which it is used in the analysis of the other three $R(+)$ heterozygotes. This is borne out by the fact that the expressivity values for the *ci ey^R* sibs of $R^{15}(+)$/*ci ey^R* flies are 2.00 for females and 2.27 for males (table 10), values which lie outside the ranges of the *ci ey^R* values calculated from all other groups of $R(+)$ tests (2.06–2.84 for females, 2.34–2.97 for males).

It is therefore more accurate to compare the values of expressivity of

$R^{15}(+)/ci\ ey^R$ not with those of their phenotypically *ci ey*^{R} sibs but with the *ci ey*^{R} values derived from the other $R(+)$ tests. The means for $R^{15}(+)/ci\ ey^R$ females vary from 2.88 to 3.20, average 3.08; and for males from 2.79 to 3.03, average 2.91 (table 10). The average expressivity for the more than 7000 *ci ey*^{R} flies from all other tests was 2.32 for females and 2.72 for males. The $R^{15}(+)/ci\ ey^R$ heterozygotes are thus more extreme than *ci ey*^{R}/*ci ey*^{R} flies, the average difference being −0.76 for females and −0.19 for males. Moreover, not only are the means for $R^{15}(+)/ci\ ey^R$ heterozygotes more extreme than the overall average for *ci ey*^{R}/*ci ey*^{R}, but the mean for the female heterozygotes lies also

TABLE 10

Means of expressivity of $R^{15}(+)$ heterozygotes and ci ey^R sibs listed in table 9.

CULTURE NUMBER	$R^{15}(+)$		*ci ey*^{R}	
	♀♀	♂♂	♀♀	♂♂
1	2.95	2.79	1.80	2.05
2	3.14	2.93	1.78	2.29
3	2.88	2.75	2.03	2.26
4	3.20	3.03	2.28	2.38
Total	3.08	2.91	2.00	2.27

beyond the whole range of all means for *ci ey*^{R}/*ci ey*^{R} females (range 2.06–2.84), while only one of the means for *ci ey*^{R}/*ci ey*^{R} males (range 2.34–2.97) surpasses, and but slightly so, the mean for the male heterozygotes.

$R^3(+)/ci\ ey^R$

Genetic Interpretation

Twelve cultures are available for an evaluation of this constitution (table 11). The following pecularities deserve discussion:

(1) All cultures deviate from equality of the two classes $R^3(+)$ and *ci ey*^{R} in the direction of a deficiency of the latter class.

(2) The deficiency of *ci ey*^{R} offspring is extreme in cultures 6–8.

(3) In addition to the flies recorded, there appeared in culture 9 one weak Minute-type male with eyeless phenotype and the most extreme expression of *ci*, class 4. Numerous black pupae occurred in all cultures which contained adult flies, unable to hatch, of phenotype Minute, eyeless, presumably of the same constitution as the single *M ey*^{R} *ci* male (the venation type cannot be ascertained in unhatched adults).

(4) The distribution of $R^3(+)$ females over the phenotypic range of vein abnormalities shows in addition to the peak at class 4 an additional peak at class 2. Normally the distribution of *ci* shows only a single maximum.

The pecularities (1), (3), and (4) are intelligible if it is assumed that the $(+)/ci\ ey^R$ P-males form not only gametes resulting from regular segregation

but also frequently undergo nondisjunction of the normal chromosome 4 and the rod shaped chromosome consisting of the base of 4 and the translocated tip of 3 (consideration of the independent chromosome unit consisting mainly of section 95D/E-97C1, and of the corresponding deficiency will be omitted). Regular segregation leads to the balanced gametes containing all rearranged [$R^3(+)$] or all normal (*ci ey^R*) chromosomes and to various unbalanced gametes all of which are deficient for sections of either chromosome 2 or 3, except for a combination consisting of normal chromosomes 2 and 3 and the base 4-tip 3 fragment as a duplication. The balanced gametes $R^3(+)$ and *ci ey^R* lead to

TABLE 11

F_1 of 12 separate cultures of 2 ci ey^R/ci ey^R ♀♀ × 1 $R^3(+)$/ci ey^R (?; see text) ♂.

CULTURE NUMBER	$R^3(+)$										*ci ey^R*									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
1	—	1	3	4	12	—	1	6	7	7	—	1	6	9	—	—	—	2	5	—
2	—	—	8	2	14	—	—	4	5	12	—	—	4	10	1	—	—	1	10	—
3	—	—	1	1	5	—	—	1	5	15	—	1	1	2	—	—	—	1	11	—
4	—	1	8	7	20	—	—	4	9	17	—	—	3	8	—	—	—	4	10	—
5	—	—	4	3	9	—	—	1	9	17	—	1	4	4	—	—	1	1	9	—
6	—	—	4	4	17	—	—	1	5	12	—	—	1	—	1	—	—	—	3	1
7	—	—	4	4	18	—	—	2	14	12	—	—	—	1	—	—	—	—	7	2
8	1	—	8	5	23	—	—	3	7	21	—	—	1	8	—	—	—	—	4	—
9	—	—	—	4	14	—	—	1	12	18	—	2	15	5	—	—	—	10	10	—
10	—	—	9	1	20	—	—	—	4	18	—	—	4	14	3	—	—	2	10	4
11	—	—	3	4	26	—	—	1	18	23	—	1	7	12	7	—	2	5	8	6
12	—	1	7	3	23	—	1	7	7	9	—	2	11	10	—	—	—	2	14	—
Total	1	3	59	42	201	—	2	32	102	181	—	8	62	83	12	—	3	28	101	13

$R^3(+)$/*ci ey^R* and *ci ey^R*/*ci ey^R* offspring in equal numbers. The unbalanced gametes do not produce viable zygotes, with the possible exception of the last named type (see below). Zygotes originating from this type are deficient for most of chromosome 4 and duplicated for the tip of 3. Each one of these two aberrations separately reduces viability greatly, and the combination is probably fatal. Possibly some of the Minute *ey^R* black pupae may be accounted for by these unbalanced genotypes, but the rest of the inviable pupae presumably owe their origin to one of the gametes resulting from nondisjunction of chromosome 4 and the rod-shaped fragment.

The nondisjunctional gametes are of four types: rearranged set and additional 4; rearranged set deficient for base 4, tip 3; normal set minus 4; and normal set plus base of 4, tip 3. The first type leads to $R^3(+)$/*ci ey^R*/*ci ey^R* offspring, the second to a lethal condition, the third to haplo-4 *ci ey^R*/o, and the last to *ci ey^R*/*ci ey^R*/dupl. (base 4, tip 3). Type I, triplo-4, $R^3(+)$/*ci ey^R*/*ci ey^R* is individually indistinguishable from the regular diplo-4 $R^3(+)$/*ci ey^R* type. It was to be expected however, that the mean expressivity of the two genotypes would be different. Since there are two peaks in the phenotypic distribution of the $R^3(+)$ females in table 11, and since the main peak falls on the most ex-

treme class, it is concluded that this class represents mostly the regular *J +)/ci ey*^{R} flies, while the secondary peak in the $R^3(+)$ females and part of e class 3 $R^3(+)$ males are regarded as due to the presence of the $R^3(+)/ci$ *ey*^{R}/*ci ey*^{R} nondisjunctional individuals. Independent proof for this interpretation will be given below. The third type of nondisjunctional zygotes accounts for at least the greater part of the Minute *ey*^{R} pupae and the single Minute *ci ey*^{R} male, since the phenotype of haplo-4 is Minute. The fourth type was not clearly distinguished among the offspring listed in table 11. Later tests showed it to be a *ci ey*^{R} duplication phenotype with low viability, shortened bristles,

TABLE 12

Means of expressivity of $R^3(+)$ *heterozygotes and ci ey*^{R} *sibs listed in table 11.*

CULTURE NUMBER	$R^3(+)$		*ci ey*^{R}		DIFF. BETWEEN MEANS	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
1	3.35	2.95	2.50	2.71	−0.85	−0.24
2	3.25	3.38	2.80	2.91	−0.45	−0.47
3	3.57	3.67	2.25	2.92	−1.32	−0.75
4	3.28	3.43	2.50	2.71	−0.78	−0.72
5	3.31	3.59	2.33	2.73	−0.98	−0.86
6	3.52	3.61	3.00	3.25	−0.52	−0.36
7	3.54	3.36	3.00	3.22	−0.54	−0.14
8	3.32	3.58	2.89	3.00	−0.43	−0.58
9	3.78	3.55	2.14	2.50	−1.64	−1.05
10	3.37	3.82	2.95	3.13	−0.42	−0.69
11	3.70	3.49	2.93	2.86	−0.77	−0.63
12	3.41	3.00	2.35	2.88	−1.06	−0.12
Total	3.44	3.46	2.60	2.86	−0.84	−0.60

trident pattern on the thorax and broad, somewhat plexus-like veins. Cytological preparations proved the expected presence of the appropriate chromosome fragment in addition to two normal sets of chromosomes.

We have thus accounted for pecularities (3) and (4) listed above. Since among the zygotes produced in consequence of nondisjunction only the $R^3(+)/ci\ ey^R/ci\ ey^R$ have good viability, these added to the regular $R^3(+)/ci$ *ey*^{R} flies increase the number of $R^3(+)$ phenotypes. Peculiarity (1), the larger number of $R^3(+)$ than of *ci ey*^{R} phenotypes in cultures 1–5 and 9–12 is thus understandable. Finally, to account for peculiarity (2), it is assumed that the P-males of cultures 6–8 were duplication types: $R^3(+)/ci\ ey^R$ plus an extra base of 4 with the tip of 3. Regular segregation of the two base 4, tip 3 fragments in these males would lead to $R^3(+)$ type offspring with or without an extra chromosome 4 and to zygotes with normal chromosomes 2 and 3, the base 4, tip 3 duplication and either one or two chromosomes 4. The "haplo-4 plus duplication" genotype would be near-lethal, the "diplo-4 plus duplication" genotype would appear *ci ey*^{R} and poorly viable. Its existence was verified cytologically.

Rare nondisjunction of the two base 4-tip 3 fragments would lead to a few typical diploid *ci ey^R/ci ey^R* offspring.

Further evidence for the interpretation of the data in table 11 comes from tests of two $R^3(+)$ females of venation class 2. By progeny tests both were proved to have been triplo-4 $R^3(+)$*/ci ey^R/ci ey^R*. It was expected that regular segregation of the two *ci ey^R* carrying chromosomes 4 would lead to $R^3(+)$ gametes containing both $R^3(+)$ and *ci ey^R*. Separate tests of 18 phenotypically $R^3(+)$ males derived from one of the two females confirmed this expectation since all proved to have carried three chromosomes 4. Finally, it was expected that $R^3(+)$*/ci ey^R/ci ey^R* flies would produce equal proportions of $R^3(+)$ and *ci ey^R* phenotypes in contrast to the deficit of *ci ey^R* offspring from $R^3(+)$*/ci ey^R* as listed in table 11. Observations of the relevant proportion in the progeny of twelve $R(+)$-3 type males agreed with this expectation. Two of these 12 males, all of which were derived from one of the original $R^3(+)$*/ci ey^R/ci ey^R* females, were identical with two of the 18 males cited above whose triplo-4 nature had been shown independently. A further analysis of $R^3(+)$*/ci ey^R/ci ey^R* flies will be offered below under the heading "heterozygotes triplogenic for the *ci* locus."

Comparison between flies heterozygous for $R^3(+)$ and *ci ey^R* and their sibs homozygous for *ci ey^R*

As shown in the preceding discussion, the $R^3(+)$ heterozygotes listed in table 11 are made up of both $R^3(+)$*/ci ey^R* and $R^3(+)$*/ci ey^R/ci ey^R* individuals. In the chapter on triplogenic genotypes it will be shown that $R^3(+)$*/ci ey^R/ci ey^R* have lesser degrees of vein interruption than $R^3(+)$*/ci ey^R*. Consequently, a mean value for the expressivity of all $R^3(+)$ flies will be lower than that from $R^3(+)$*/ci ey^R* alone. Most of the *ci ey^R* sibs are *ci ey^R/ci ey^R*, but a few of them may, in addition, contain the base 4-tip 3 duplication. An effect of this duplication on the mean expressivity, if it exists at all, is slight. It seems to involve a small shift toward greater expressivity since the mean values for the *ci ey^R* types of cultures 6–8 are higher than those for most other cultures listed in table 11. Many of the values for the other cultures themselves are higher than the values for *ci ey^R/ci ey^R* flies raised in simultaneous experiments involving other $R(+)$ alleles in which diplo-4 flies only were expected (*cf.* the expressivity values listed in tables 6 and 7).

The overall means of expressivity for each phenotype are listed in table 12. For the $R^3(+)$ females the means for separate cultures vary between 3.25 and 3.78, with an overall mean of 3.44. In contrast, the separate means of the *ci ey^R* sibs vary between 2.14 and 3.00, with an overall mean of 2.60. In every culture the difference in mean value of expressivity between $R^3(+)$ and *ci ey^R* females is negative, with an overall difference of −0.84. In other words, the position-allele heterozygotes are on the average nearly one class more extreme than the mutant homozygotes. For the males the same general picture holds. Individual means for $R^3(+)$ males vary between 2.95 and 3.82, for *ci ey^R* sibs between 2.50 and 3.25. Again in every culture the mean value of expressivity for the $R^3(+)$

males is larger than for the *ci ey*^{R} males, the overall difference being −0.60.

The differences for both females and males represent minimum values for the comparison of *R*^{3}(+)/*ci ey*^{R} versus *ci ey*^{R}/*ci ey*^{R} since, as pointed out in a preceding paragraph, the true value for *R*^{3}(+)/*ci ey*^{R} heterozygotes is greater, and the true values for *ci ey*^{R}/*ci ey*^{R} smaller than those listed for the *R*^{3}(+) and *ci ey*^{R} phenotypes.

HETEROZYGOTES TRIPLOGENIC FOR THE CI LOCUS

As has been discussed above, two of the *R*(+) alleles—namely, *R*^{3}(+) and *R*^{15}(+)—can be obtained in the constitution *R*(+)/*ci ey*^{R}/*ci ey*^{R}. *R*^{3}(+)/*ci ey*^{R}/*ci ey*^{R} flies are heterozygous for the *R*^{3}(+) rearrangement and, in addition,

TABLE 13

F$_1$ *of ci ey*^{R}/*ci ey*^{R} ♀♀ × *R*^{3}(+)/*ci ey*^{R}/*ci ey*^{R} ♂♂.

	R^3(+)										*ci ey*^{R}									
	♀♀					♂♂					♀♀					♂♂				
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Total	1	23	25	9	—	—	12	33	9	—	—	7	21	23	2	—	—	28	45	3
Means of expressivity	1.73					1.94					2.38					2.67				
Diff. between means	♀♀: +0.65; ♂♂: +0.73																			

contain an extra chromosome 4. They are thus triplo-4 in constitution and have normal, not eyeless, eyes. *R*^{15}(+)/*ci ey*^{R}/*ci ey*^{R} in contrast are diploid except for the duplicating insertion of section 101F1-102C4 of chromosome 4 into 3R. This insertion contains the *R*(+) allele but not the *ey* locus. Consequently the flies are triploid for the *ci* locus ("triplogenic") but have *ey* eyes.

R^3(+)/*ci ey*^{R}/*ci ey*^{R} triplo-4 are more nearly normal than *R*^{3}(+)/*ci ey*^{R} diplo-4 individuals. This was deduced earlier from a consideration of types of offspring to be expected from *R*^{3}(+)/*ci ey*^{R} heterozygotes and the bimodal distribution of the *R*^{3}(+) phenotypes recorded in table 11. There is also independent proof for this statement. In crosses of *R*^{3}(+)/*ci ey*^{R}/*ci ey*^{R} males to *ci ey*^{R}/*ci ey*^{R} females most of the *R*^{3}(+) offspring are triplo-4 and the *ci ey*^{R} are diplo-4. Data from three such crosses are given in table 13. The mean of expressivity for *R*^{3}(+) triplo-4 females is 1.73, for males 1.94. The values for *ci ey*^{R} diplo-4 sibs serve as controls; they are 2.38 and 2.67, respectively. A comparison between the *R*^{3}(+) and *ci ey*^{R} values shows that the *R*^{3}(+)/*ci ey*^{R}/*ci ey*^{R} flies are more nearly normal than the *ci ey*^{R}/*ci ey*^{R} sibs, the differences being significant and positive for females and males alike—namely, +0.65 and +0.73. It is seen that triplo-4 *R*^{3}(+) flies are not only more nearly normal than diplo-4 *R*^{3}(+) but even more nearly normal than diplo-4 *ci ey*^{R}.

An identical result is obtained from the comparison of *R*^{15}(+)/*ci ey*^{R}/*ci ey*^{R} versus *ci ey*^{R}/*ci ey*^{R} versus *R*^{15}(+)/*ci ey*^{R} phenotypes. In a preceding section it had been shown, that *R*^{15}(+)/*ci ey*^{R} are more extreme than *ci ey*^{R}/*ci ey*^{R} flies.

It had also been pointed out that the *ci ey*^{R} sibs of the $R^{15}(+)/ci\ ey^R$ individuals were a mixture of about equal numbers of two genotypes—namely, $ci\ ey^R/ci\ ey^R$ and $R^{15}(+)/ci\ ey^R/ci\ ey^R$—and that the mean expressivity calculated jointly from the two genotypes was smaller than that of $ci\ ey^R/ci\ ey^R$ flies in general. It is concluded that this shift toward normality of the *ci ey*^{R} phenotypes in $R^{15}(+)$ cultures is due to the triplogenic $R^{15}(+)/ci\ ey^R/ci\ ey^R$ flies, which thus are more nearly normal than $ci\ ey^R/ci\ ey^R$, which in turn are more nearly normal than $R^{15}(+)/ci\ ey^R$.

DISCUSSION

Position effects are recognized by changed action of genes in new positions. How this changed action is produced is not subject to direct investigation. It may be hoped, however, that data on the results of changed positions might constitute limits within which any acceptable interpretation of the causes of changed genic action will have to lie. The following discussion is an attempt in this direction.

Position effects may be thought of as either changes of genes themselves which in turn lead to changes in the immediate gene product or as changes of the gene product only. If the first is the case, the work of STURTEVANT (1925), DUBININ and SIDEROW (1935) and others has shown that the gene changes in altered positions, unlike those of typical mutations, are reversible with restoration of the original position. In the second case, that of an unchanged gene, but with changed product, two alternative possibilities may be envisaged. Either the quality of the product controlled by a position allele may differ from that of the normally located allele, or it may be the quantity of the product. In either case, it is clear that the site of the primary event is in or at the chromosome, since the changed effect is caused by the changed position within the chromosome. Whether or not genic action occurs by means of "plasmagenes" (cf. WRIGHT 1945) outside the nucleus, the original genic action which would be responsible for the production of such hypothetical units would still take place at the chromosomal gene locus.

In an earlier paper (STERN and HEIDENTHAL 1944) it was shown that certain position effects observed at the *ci* locus could be interpreted in either one of two basically different ways. On the one hand, it was possible to assign lower efficiency values in terms of results of gene-substrate interaction to the position alleles than the efficiency values of the alleles in their normal positions. On the other hand, it could be assumed that both the gene and the substrate for its immediate action remained qualitatively unchanged but that the quantity of the available substrate in the altered position was decreased. The result of such a situation would be a change in amount of immediate gene product. These alternative interpretations will now be applied to the new findings reported in the present paper. Two of these are:

(1) Heterozygotes between certain position alleles $R(+)$ and the mutant allele *ci* are phenotypically more extreme than either of the two homozygotes.

(2) Triplogenic heterozygotes of the type $R(+)/ci/ci$ are intermediate between the two diplogenic heterozygotes.

The first of these two results is nearly, if not completely unique. Genetic nomenclature has no term to designate the phenomenon of a heterozygote's being neither intermediate, nor additive, nor dominant in relation to the homozygotes. A new term "ultra-dominance" could designate the new relation were it not for the fact that the word "dominance" or any term containing this word implies the often mistaken idea of an antagonistic action between two alleles of which one, the "stronger," is dominant.

The second of the two results summarized above is equally unusual if considered in combination with the first. Since the hemizygotes of the four $R(+)$ alleles lead to normal phenotypes, result (1) may be expressed in the sentence: "Addition of a *ci* allele to $R(+)$ shifts the phenotype from normal to less nearly normal than *ci/ci*." Correspondingly, result (2) would have to be phrased: "Addition of a second *ci* allele shifts the phenotype back to more nearly normal than *ci/ci*."

An attempt to endow the four position alleles $R^{7}(+)$, $R^{3}(+)$, $R^{12}(+)$, and $R^{15}(+)$ with changed attributes, as compared to the +-allele, does not only require that these attributes are reversible with reversed position changes, but that they are of a unique nature as expressed in their interrelations with the recessive *ci* allele of the same locus.

In former papers, differences between different alleles at the *ci* locus have been interpreted in terms of different powers, c, of the alleles to combine with a cellular substrate, S, and of different efficiencies, e, of converting S into a product, P, which acts toward normal venation. In order to measure relative values for these genic attributes, it was assumed that different amounts of P are so correlated with phenotypic effect that the effect either increases with increasing P, or having reached an upper limit, remains constant. An interpretation on these premises of the results reported in this paper leads to the following considerations.

Since

$$\mathrm{P}(R(+)/ci) < \mathrm{P}(ci/ci) \qquad (1\,a)$$

it is concluded that

$$c_{R(+)}\, c_{R(+)} < c_{ci} e_{ci} \qquad (1\,b)$$

Since, however,

$$\mathrm{P}(R(+)/R(+)) > \mathrm{P}(ci/ci) \qquad (2\,a)$$

and also

$$\mathrm{P}(R(+)/M-4) > \mathrm{P}(ci/M-4) \qquad (2\,a^{1})$$

it is concluded that

$$c_{R(+)}\, c_{R(+)} > c_{ci} e_{ci} \qquad (2\,b)$$

Deductions (1 b) and (2 b) are incompatible. It follows that the theoretical premises are insufficient to explain the data.

It might be suggested that the complicated facts related to the $R(+)$ alleles

could be accounted for if the relation between quantity of P and phenotype were represented by a more complex curve than one which increases to and remains at an upper limit. If we consider the two alleles, + and *ci* and, discarding the analysis into c and e attributes, assign to each a single value of activity, as for instance three for *ci* and ten for +, we would expect increasing activity in the series $1ci=3$, $2ci=6$, $3ci=9$, $1+=10$, $+/ci=13$, $2+=20$, $3+=30$. Actually the phenotypic approach to normality corresponds to the postulated values for $1ci$, $2ci$, $3ci$ and $1+$. However, the phenotype of $+/ci$ drops significantly even if only slightly below that of +, with the phenotypes of $2+$ and $3+$ rising again to complete normality. Values assigned to the $R(+)$ alleles discussed in this paper and based on the phenotype of the hemizygotes would have to be between nine and ten. The phenotypes of the combinations $R(+)/ci$ with activity values expected from cumulative action values of between 12 and 13 lie in reality below those of the phenotype ci/ci with its value of six. In other words, the relation between activity values and observed phenotypes would have to be represented by a curve rising to a maximum, then dropping deeply and again rising to an upper limit. Consideration of other alleles and combinations would add to the complexity. While such involved relations are not unknown in physiological phenomena, their *ad hoc* introduction for genic action appears not very satisfactory.

Having rejected interpretations in terms of altered c and e attributes of the position alleles and in terms of complex curves for gene activity versus phenotypic expression, we proceed now to the ***assumption of reduced amount of available substrate.*** If this hypothesis is made, facts reported by STERN, SCHAEFFER, and HEIDENTHAL (1946) require a specification within the framework of the hypothesis. It was shown that on the average heterozygotes of the type $R(+)/ci$ are less nearly normal in phenotype than heterozygotes of the type $R(ci)/+$. Since a reduction of available substrate would on the average amount to the same quantity in $R(+)$ and $R(ci)$ rearrangements, it follows that the + and *ci* alleles of the heterozygotes do not share the substrate independently of their position. The facts become intelligible if the ***normally located allele has an at least partial priority on the substrate, S, with the position allele sharing S to a secondary degree.*** The hypothesis might be represented by a diagram (fig. 2): a field is delimited inside of which the normally located allele and outside of which the position allele is placed. In figures 2 a–e homozygous, hemizygous, and heterozygous combinations of the normally located allele + and *ci* are depicted in order of decreasing normality of phenotype. Figures 2 f–l shows various constitutions involving position alleles. Since different position alleles have different effects, under the hypothesis assumed to be due to individually different degrees of reduced available substrate, a special diagram would be required for each position allele. The various degrees of reduced substrate could be illustrated by varying the distance between the position allele and the region of normal location of the *ci* alleles. Since the present communication does not attempt to analyze in detail the variability of effects of different position alleles, a constant distance of the position allele

from the region of normal location has been employed in all the diagrams, except in figure 2 h.

The various constitutions involving position alleles will now be discussed. In the case of *R(ci)*/+ (fig. 2 f), the + allele in its normal position obtains sufficient S to transform it by means of the allele's high c and e attributes to a product resulting in nearly normal venation, with the *ci* allele in its changed position being a secondary competitor. The combined action does not deviate

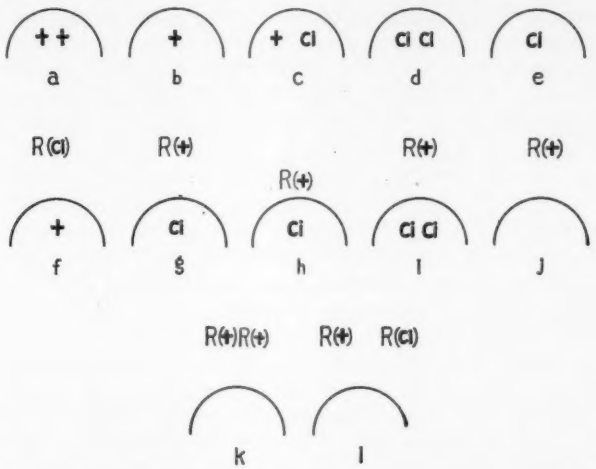

FIGURE 2.—Diagrams of the location of alleles in their normal position within a field of high concentration of substrate and of the location of position alleles outside of the field. For details see text.

much from that of a nonposition, heterozygote +/*ci* (fig. 2 c). In case of *R*(+)/*ci* (fig. 2 g, h), the *ci* allele in its normal position obtains sufficient S as does the normally located + allele in the previous case but, due to the *ci* allele's low efficiency (e_{ci}) produces a product that results in low degrees of venation only. The +allele in its abnormal position, acting in its secondary capacity, has at its disposal only the smaller amount of S not blocked by the priority of *ci*. In 15 out of 19 *R*(+) alleles investigated in the heterozygote *R*(+)/*ci* (fig. 2 h) the secondary contribution of the +allele with its high e value added to the primary contribution of the *ci* allele results in a total action which is more nearly normal than homozygous *ci*/*ci*. In the four *R*(+) alleles (fig. 2 g) which form the basis of the present study so small an amount of S is left for the position-alleles, that the combined effect of *R*(+) and *ci* remains below that of *ci*/*ci*.

Similarly, in the triplogenic constitutions *R*(+)/*ci*/*ci* (fig. 2 i) the two normally located *ci* alleles with their priority on S produce an effective amount of P not unlike that in a *ci*/*ci* homozygote, and the *R*(+) position allele adds

further toward normality of venation. The result is the shift in phenotype from the extreme abnormality $R(+)/ci$ to the type of venation $R(+)/ci/ci$ which is intermediate between the two diplogenic heterozygotes.

The hypothesis developed here accounts also for the fact that hemizygotes of the $R(+)$ alleles (fig. 2 j) lead to normal phenotypes. In these cases there is no primary allele present in a normal position which would obtain a priority on S. As a result, the position allele $R(+)$ is provided with a sufficient amount of S. The same reasoning applies to the homozygote $R^{15}(+)/R^{15}(+)$ with its normal phenotype (fig. 2 k). Additional discussion is needed, however, in order to account for the *ci* phenotype of $R^{2}(+)/R^{2}(+)$. Such discussion might proceed on similar lines as that offered on the fact that hemizygotes for the normally located ci^{w} allele show normal veins, but abnormal legs and bristles, while homozygotes for ci^{w} possess abnormal venation, but normal legs and bristles (STERN and SCHAEFFER 1943a). These facts regarding ci^{w} were interpreted in terms of two different reactions competing for the same substrate, and assuming that only one of these reactions contributed toward normal venation. In the case of $R^{2}(+)$ it might be likewise postulated that the reduction of S available to the position allele is not due only to its unfavorable position but also to competitive removal of S at its normal position by the translocated chromosome 3R. A more detailed treatment together with additional data will be presented at a later date. Altogether, under these assumptions a position effect is regarded as dependent upon (1) the changed position of the gene whose effect is studied, (2) the specific allele of the gene concerned, and (3) the type of translocated chromosomal material which has taken the original place of the position allele.

The hypothesis of reduced S and priority of a normally located allele sheds light on a result reported earlier (STERN and HEIDENTHAL 1944): heterozygotes between +- and *ci*-position alleles, $R(+)/R(ci)$ (fig. 2 l), are less abnormal in venation than $R(+)/ci$ heterozygotes. This was unexpected since $R(ci)/ci$ leads to a lesser degree of venation than ci/ci. It had been supposed therefore that substitution of $R(ci)$ for *ci* in $R(+)/ci$ would also lead to greater abnormality instead of the observed opposite effect, an approach toward normality. Under the assumption of priority on S for the typically located *ci* allele, but lack of priority for the $R(ci)$ allele, the observed result of $R(+)/R(ci)$ could have been predicted: lack of priority hinders the $R(ci)$ allele with its low efficiency from depriving the $R(+)$ allele of as much S as the *ci* allele would have done.

There is, however, one property of the $R(+)/R(ci)$ heterozygotes which does not follow from our premises. This is a relation according to which those of the five $R(ci)$ alleles tested which cause an extreme *ci* phenotype in $R(ci)/+$ produce the least extreme expression in $R(ci)/R(+)$. Further data are accumulating which add to the information on position effects at the *ci* locus. It will remain for the future to evaluate whether the sum of all data supports the hypothesis presented, how much modification of the hypothesis is needed, or whether it will have to be abandoned altogether.

The somewhat formal hypothesis of the mechanism of position effects at the *ci* locus will finally have to be translated into terms of cellular physiology. The assumptions made do not imply random distribution of the postulated substrate throughout the nucleus, but rather some kind of diffusion of S from a region of higher concentration. This region is near to the normal locus of the + or *ci* allele. The assumption of priority on S for the normally located allele with its implied blocking of S on its way to the position allele may be interpreted in terms of preferential, or exclusive distribution of S along the chromosomes from its region of high concentration. It is a fact that the majority of the striking position effects at the *ci* locus are correlated with a removal of the allele from the heterochromatic proximal region of chromosome four. Does this region provide the substrate for the cubitus alleles, and does this substrate by means of the common chromocenter reach the position alleles in their new regions within, or at the distal ends of other chromosomes? Are these assumptions compatible with our knowledge of physiological processes occurring in very small volumes and perhaps involving very small numbers of molecules?

It may be recalled that the position-allele $R^2(+)$ occupies its normal locus in an unchanged, right arm of chromosome four. The associated translocation has occurred in the left, heterochromatic arm and resulted in an attachment of most of chromosome 3R to 4L. Cases like this may, at a later occasion, serve to define more closely the factors involved in the priority hypothesis.

In order to account for position effects, the fact of somatic pairing of chromosomes in the Diptera has been used at various occasions (for example, STURTEVANT 1925; MULLER 1935; EPHRUSSI and SUTTON 1944; STERN and HEIDENTHAL 1944; GERSH and EPHRUSSI 1946). The present hypothesis, while still referring to the somatic association of heterochromatin within the chromocenter, does not require somatic pairing in heterozygotes, involving position alleles. This is an advantage, since in some cases of position effects cytological data do not support the assumption of somatic pairing in heterozygotes between position alleles and normal alleles. Thus, in $R^3(+)/ci$ no cases have been observed in salivary gland nuclei in which the $R(+)$ locus, translocated to the tip of the long rearranged 2L-3L-4R autosome, was paired with the normal chromosome four, located at the chromocenter, nor was the $R^{15}(+)$ allele, inserted in 2L, ever seen to pair with the normal chromosome four. A few early mitotic prophases in $R^3(+)/ci$ individuals likewise showed the normal chromosome four well removed from that region of the long compound autosomal arm —namely, its tip—which contains the homologous translocated section of the fourth chromosome. While the conditions in the salivary gland or oogonial nuclei may not necessarily be like those in the cells responsible for the venation effect, we regard the inference from one to the other as reasonable.

SUMMARY

Out of 19 position alleles, $R(+)$, in which a chromosomal rearrangement had taken place close to the locus of the +allele of the recessive mutant gene cubitus interruptus (*ci*) of *Drosophila melanogaster*, four $R(+)$ alleles showed

the following peculiarity: Both female and male heterozygotes $R(+)/ci$ exhibit a more extreme degree of interruption of wing veins than the homozygote ci/ci.

Hemizygotes of the constitution $R(+)/M\text{-}4$ for all four $R(+)$ alleles, designated as $R^2(+)$, $R^3(+)$, $R^{12}(+)$, and $R^{15}(+)$, are normal in phenotype, with probably a slightly greater tendency for weak degree of *ci* venation than in $+/M\text{-}4$.

Homozygotes for $R^3(+)$ and $R^{15}(+)$ are not viable. $R^{12}(+)/R^{12}(+)$ appear normal, $R^2(+)/R^2(+)$ similar to ci/ci.

The heterozygotes $R(+)/ci$ for all four $R(+)$ alleles are more extreme than the $R(+)$ hemizygotes and the *ci* homozygotes. The heterozygotes between $R^2(+)$ or $R^{12}(+)$ and *ci* are more extreme than either of the two homozygotes.

Triplogenic heterozygotes of the types $R^3(+)/ci/ci$ and $R^{15}(+)/ci/ci$ were obtained. They are intermediate in phenotype between the two diplogenic heterozygotes.

Cytologically the rearrangements $R^2(+)$ and $R^{12}(+)$ involve a break in the right arm of chromosome 4 between the *ci* locus and the kinetochore. $R^3(+)$ involves a break in the left arm of chromosome 4. $R^{15}(+)$ consists of an insertion of a section of chromosome 4, right arm including the *ci* locus into chromosome 2.

The properties of the $R(+)$ combinations cannot be accounted for satisfactorily (a) by assigning combining powers, c, and efficiencies, e, to the position alleles different from the c and e attributes of the normally located + allele, nor (b) by assuming a complex relation between varying quantities of gene product and phenotype.

A hypothesis is developed according to which the attributes of position alleles remain unchanged whereas the amount of substrate, S, available to a position allele is reduced as compared to that present in the original position. This hypothesis demands the specification that in heterozygotes the normally located allele has an at least partial priority on S, with the position allele sharing S to a secondary degree. Altogether, a position effect is regarded as dependent upon (1) the changed position of the gene whose effect is studied, (2) the specific allele of the gene concerned, and (3) the type of translocated material which has taken the original place of the position allele.

The proposed hypothesis does not require somatic pairing in heterozygotes involving position alleles.

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MUTATION OF BACTERIOPHAGE WITH RESPECT TO TYPE OF PLAQUE

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ONLY a few instances of well defined mutation have been described for the bacteriophages. The best known form a class of mutations affecting host range, recently studied by LURIA (1945a, 1945b), whose papers may be consulted also for mention of the earlier literature.

This paper is concerned with a second class of mutations, occurring within single plaques of certain coli-dysentery phages, where the appearance of the mutant type occasionally gives rise to variegated plaques. From the variegated plaques, the mutant is readily isolated by virtue of its distinctive plaque type and proves to differ from the parent type in causing prompt rather than delayed lysis in undiluted culture, but to be nearly identical with it otherwise in growth characteristics, host range, and antigenic specificity.

The new mutation has been described in a preliminary note (HERSHEY and BRONFENBRENNER 1945), and the existence of the two types of phage ("T4a" and "T4b") in lysates has been mentioned by DEMEREC and FANO (1945). A series of mutations affecting plaque type, which like ours was not related to adaptation to resistant bacterial strains, was noted also by SERTIC (1929). Its relation to the mutation described here is not clear from the published description.

NOTATION

The system of naming mutations of bacteriophages to be used in this paper departs somewhat from systems previously used. It has been developed during the past year by a group of interested workers (M. DEMEREC, M. DELBRÜCK, S. E. LURIA, A. H. DOERMANN, and the writer) in an attempt to combine convenience and descriptiveness within the usual genetic convention. It is to be hoped that it is a step toward a uniform nomenclature for the bacterial viruses.

Wild type phages are to be designated, following the notation of DEMEREC and FANO (1945), by numbers preceded by the letter *T* (for type)—for example, *T1*. The letter *T* may further be taken as a generic designation for phages acting on *Escherichia coli* strain *B* (and, so far, also on many strains of Shigella). The numbers are arbitrary, with the single restriction that the even numbers should be reserved for the antigenically and morphologically related phages (DELBRÜCK 1946a) already represented by *T2*, *T4*, and *T6*. These are homologous with the serological group II of BURNET (1933a), the "WLL" of SCHLESINGER (1934), and the "PC" of BRONFENBRENNER (KALMANSON and BRONFENBRENNER 1939). They evidently form a very commonly studied group among the coliphages. As will be seen presently, they also have functional and mutational properties in common.

At present the numbers *T1* through *T7* are permanently occupied (DEMEREC and FANO 1945). For the purposes of the present study, we are adding an additional wild type (the "AC" of BRONFENBRENNER's collection), which we shall call *T16*.

The derivatives of *T2* to be mentioned in this paper will serve to illustrate the new system of naming mutants of the bacterial viruses.

It sometimes happens that a given type of phage gives rise spontaneously during propagation to a line differing from its progenitor. When this happens, one has the choice of throwing it away, giving it an arbitrary designation, or working out its mutational relationships. Since the first is not always desirable, and the last not always possible, it is necessary to have some system for designating phages which are neither authentic wild types (that is, phages certainly not nearly related to other known phages), nor mutations obtainable at will. For this type of variant, and where the study of host relationships and antigenic properties supports the inference as to generic relatedness, we use capital letters to indicate the subtypes. For instance, the three lines of putative PC phage established by KALMANSON, LURIA and HERSHEY prove to be genetically different, but certainly very closely related. These will be called *T2K*, *T2L*, and *T2H*, respectively, and will be described further below. For the present, owing to the nature of the material, these symbols are not likely to be confused with the notation for the dominant genetic factors of classical genetics.

For authentic, independent genetic factors, we shall use small letters descriptive of the observed mutation away from wild type. Two of these will be mentioned here.

One class of mutants are those characterized by altered host range, the prime mutants in the notation of LURIA (1945a). These have usually been isolated by means of specifically resistant variants of the sensitive bacterial strains. (The nomenclature for the bacterial mutants themselves is illustrated by the example *R/3,2,4,7*, which means a derivative of *R* selected for resistance to *T3*, proving to be resistant also to *T2*, *T4*, and *T7*, but to no other virus of the *T* system (DEMEREC and FANO 1945)). Mutant phages, differing from the parent type by their ability to infect bacteria resistant to it, will now be designated by the letter *h* (for the altered host range), followed when necessary by the symbol in parentheses for the bacterial strain which served for the isolation. Thus *T2H* plated on *R/2H* yields *T2Hh*(*R/2H*). Among these there are two varieties: h^t (for turbid plaques) and h^c (for clear), which differ both in appearance of plaques on *R/2H*, and in infectivity for this host relative to that for the fully sensitive bacterium. It is not yet known whether h^c and h^t are separate loci.

The new mutation, corresponding to a second genetic factor, will be designated by the letter *r* (for rapid lysis)—that is, *T2Hr*. When *T2Hh* undergoes *r* mutation, we get *T2Hhr;* when *T2Hr* undergoes *h* mutation, we get *T2Hrh*. If it is desired to specify the wild type alleles of these characters, we use $T2Hr^+$, $T2Hh^+$ or $T2Hr^+h^+$. Usually the symbol *T2H* alone will identify wild type explicitly enough.

MATERIALS

Bacterial strains

Our stock of sensitive *E. coli* (the PC of BRONFENBRENNER) differs from the strain *B* derived from the same source by DELBRÜCK and LURIA (1942) by at least one mutation, since it is sensitive to a killer substance (cf. GRATIA 1925) produced by another strain of *E. coli* to which *B* is resistant. We therefore designate our stock by the letter *R* (for rough). We prefer *R* to *B* for mutational studies owing to the usefulness of the additional marker, which proves to be independent of virus sensitivity. Another derivative of the same culture, called *S* (for smooth), we prefer to *R* for propagation of phage stocks and for plaque counts, since *S* grows to higher populations in broth and provides a whiter background in seeded agar plates. A fourth derivative of the same culture, known as *H*, originally selected for its tendency to yield sterile lysates with *T2H*, proves to adsorb this phage better than does *R*, and is therefore used in some of the experiments cited below. It seems necessary to record these details, which serve at least to call attention to the difficulty of preserving uniform biological materials. In general, however, the *T* system of phages act identically on the bacterial strains mentioned, so that the casual reader may consider the terms *R*, *S*, *H*, *B*, and "sensitive bacterium" as synonymous.

New phages

The new strains of phage mentioned in this paper originated as follows:

T16. A line stemming from a single plaque of the "AC" phage of BRONFENBRENNER.

T2H. A line established by the writer, stemming from a single plaque of the "PC" phage of BRONFENBRENNER. This line has been described as "P9H" (HERSHEY, KALMANSON, and BRONFENBRENNER 1944).

T2K. A line established by KALMANSON, stemming from a single plaque of the same "PC" phage, otherwise called "PC" (KALMANSON and BRONFENBRENNER, 1939).

T2L. The phage gamma of DELBRÜCK and LURIA (1942) was established by them from a single plaque of *T2K* and will now be called *T2L* to distinguish it from the lines mentioned above. This strain is the original *T2* (DEMEREC and FANO 1945). We also have obtained a phage similar to or identical with *T2L* from *T2K*.

The three lines of *T2* breed true on sensitive bacterial strains. It is not known certainly that they have a recent common parent, though this is very probable in view of their origin and close similarity. At any rate they are not known to represent "wild types" of remotely independent origin, and the arbitrary notation suggested above serves to indicate this fact, until their mutational relationship has been worked out.

The differentiation of the even-numbered phages can be made by means of the following mutants (among others, to be described elsewhere) isolated by us from the sensitive bacterial strain: (*R/2H*); (*R/2H*, *2K*); (*R/2K*, *6*);

(R/2H, 2L); R/2H, 2K, 2L; R/6, 16; R/3, 4, 7, 16. The mutations indicated in parentheses are rare.

The close relationship between the *T2* lines is shown by the fact that the great majority of bacterial mutants resistant to any one are resistant to all, and by the inability of anti-*T2K* immune serum to distinguish them clearly.

The remoter relationship among the wild types *T2*, *T4*, *T6*, and *T16* is shown by the fact that all cross-react in antisera to any one, but with unmistakable quantitative differences (M. DELBRÜCK, personal communication, and unpublished experiments of the writer). Our classification of the even-

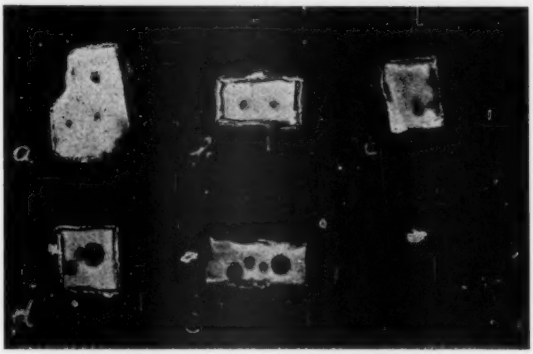

FIGURE 1.—Plaques illustrating *r* mutation. a. Plaques of *T2K*, wild type, and a mottled plaque arising from a bacterium infected with both wild type and *r* mutant. b. Two plaques of *T2K* wild type. The one to the left shows a typical sector of *r* mutant. c. Two contiguous plaques of *T2K* wild type. The lower one shows a very large sector of *r* mutant. d and e. Plaques from a mixture of pure stocks of *T2H* wild type and *r* mutant.

numbered phages is thus in accord with the data of BURNET (1933a), which show that wild type phages of a given serological group are nearly always neutralized more rapidly by homologous antiserum than by antiserum to related types.

ISOLATION OF THE *r* MUTANT

Plaques of *T2H* in very soft agar (that is, 0.6 percent "Difco standardized") in plates prepared by the agar layer method (HERSHEY, KALMANSON, and BRONFENBRENNER 1944) show a distinct halo of partial lysis visible at 18 to 24 hours. About one percent of the plaques show sectors of more or less complete lysis within the halo. Phage propagated from the aberrent sectors invariably yields, in addition to the parent type, *r* plaques of somewhat larger size, which differ in showing nearly complete lysis at the periphery. There is usually an intermediate ring of imperfect lysis in the *r* plaques. On further isolation, both types breed true except for further *r* mutations. In addition to the variegated plaques, platings of stocks of wild type may or may not show a few primary *r* plaques when practicable samples are plated. The proportion probably de-

pends on whether *r* mutations have occurred earlier or later during lysis.

Figure 1 shows photographs (made by exposing sensitized paper to light transmitted through slices of agar cut out and supported on cover slips) of plaques of wild type and *r* mutant. The contrast between the two types of plaque is especially well shown in the plating of a mixture of the two phages. The sharp boundary between overlapping plaques of the two kinds shows that the *r* mutant cannot lyse bacteria already inhibited by wild type.

Under slightly different conditions of plating, where the wild type plaques may lack the halo, the *r* plaques may show one (as found by DEMEREC and FANO 1945). In platings on the surface of one percent agar plates, neither type shows a halo, and there is only a difference in average size of plaques.

PARALLEL MUTATIONS

The *r* mutation occurs in an identical manner in all the phages so far examined which belong to the even numbered serological group—namely, the three lines of *T2* itself, several *h* mutants of these, and *T4*, *T6*, and *T16*. The only difference we have noted is that halo formation with wild type is more sensitive to conditions of plating with some phages than others. The halo is especially well marked with *T2H*. With this phage, and with *T4* and *T6*, the contrast between plaques of wild type and *r* mutant is very striking.

The even-numbered phages comprise a group of antigenically related small-plaque formers, whose morphology, as revealed by the electron microscope, is identical within the group (*T2L*, *T4*, and *T6*) (DELBRÜCK 1946a), but is easily distinguishable from that of unrelated phages. In addition, they have in common a peculiar lysis-inhibiting property, very noticeable in cultures made for production of phage stocks, where visible lysis ensues only some five or six hours after infection of all the bacteria with phage. This behavior stands in sharp contrast with the latent period of phage growth at high dilutions of bacteria and phage, which is only 21 to 26 minutes for several of these phages (DELBRÜCK 1946a, and unpublished experiments of the writer). According to experiments in progress of A. H. DOERMANN and M. DELBRÜCK (personal communication), it is likely that the delay is due to reinfection of the bacteria with phage liberated by the lysis of a few bacteria, which somehow interferes with the lysis of the remainder. The *r* mutants uniformly differ in causing complete lysis in about one hour under identical conditions. The other members of the *T* system (odd numbered from *T1* to *T7*, inclusive) do not cause lysis-inhibition and do not show a comparable mutation.

INDEPENDENT MUTATIONS

The occurrence of parallel mutations in several closely related strains of phage suggests at once that the same phage can undergo two or more mutations independently, for it is likely that the strains themselves differ from each other by one or more mutations. This supposition is confirmed by showing that the known *h* and *r* mutations of *T2H* correspond to independent genetic factors.

Mutants active on the bacterial strain *R/2H* (resistant to *T2H*) are isolated from *T2H* by plating about 5×10^8 particles of this phage in agar seeded with the resistant bacterium. Two types of mutant are obtained, both regularly appearing with about the same frequency in initially pure stocks of *T2H* and both breeding true on sensitive bacteria. These will be called *T2Hh*^{t} and *T2Hh*^{e}. *T2Hh*^{t} produces very faint plaques on *R/2H*, and only about 20 percent as many on this bacterium as on the sensitive strains. *T2Hh*^{e} produces clear plaques to a count of 80 percent or so of the titre on sensitive bacteria. Both produce plaques indistinguishable from those of *T2H* on *S*. Both undergo *r* mutations, yielding *T2Hh*^{t}*r* and *T2Hh*^{e}*r*, which have about the same relative infectivity as the corresponding *r*$^+$ forms, but produce typical *r* plaques.

T2Hrh^t and *T2Hrh*^{e} can also be isolated from *T2Hr* by selecting the *h* mutants, and the resulting phages seem to be identical with those obtained through the reverse mutational sequence.

If the *h* and *r* mutations are truly independent of each other, the rates of the parallel mutations ought to be the same. The rates of *r* mutation cannot be measured conveniently, because we have no method for counting small numbers of *r* mutants in the presence of an excess of wild type. The rates of the *h* mutations can be measured, however, subject to certain technical difficulties.

The rate problem is essentially the same for phage as for bacteria, and our understanding of it is owing entirely to the work of LURIA and DELBRÜCK (1943) on bacterial mutation. A purely formal difference from their analysis is required because of our lack of knowledge of the mode of intracellular growth of phage (cf. LURIA 1945a). If we define the chance of a mutation occurring among N phage particles during the population increment dN by

$$a\,dN, \tag{1}$$

so that a is the probability of mutation per phage particle per act of duplication, a is a suitable measure of mutation rate, and its experimental determination requires no assumption about the nature of growth or mutation. From (1) we get

$$a = \frac{-\log_e P_0}{N - N_0}, \tag{2}$$

which is equivalent to the equations (4) and (5) of LURIA and DELBRÜCK (1943). Similarly, from the differential equation for the growth of mutants

$$dh = a\,dN + \frac{h}{N}\,dN, \tag{3}$$

in which a dN is the contribution from the mutations and (h/N) dN is the contribution from the growth of progeny of previous mutations, to the total increment of mutants dh, we get

$$h = aN \log_e(NCa), \tag{4}$$

identical with their equation (8). If the mutant phage are enumerated with an efficiency E less than unity, one uses instead of (4)

$$h = aEN \log_e(NCa). \qquad (5)$$

These equations are subject only to the restrictions stated by LURIA and DELBRÜCK (1943). We now discuss their use in estimating rates of mutation of phage by two methods.

In the first method, a number of samples of phage, starting from amounts N_0 too small to contain any mutant progeny, are propagated in separate cultures of susceptible bacteria, the amounts and conditions of lysis being chosen so that only a fraction of the cultures will yield any mutants at all. After allowing the cultures to lyse, determining the average total number of phage particles N per lysate from a few tubes, and plating the entire contents of the remaining tubes with bacteria resistant to the parent phage, one determines the mutation rate a by equation (2), in which P_0 is the fraction of tubes yielding no mutants.

This method has the advantage that the relative growth rates of parent and mutant phage need not be known. It has the practical disadvantage that the method fails unless P_0 falls between, say, 0.3 and 0.9, which requires a careful choice of conditions of lysis. Its use is also restricted by the requirement that mutant phage be detectable with nearly perfect efficiency, for (2) is not a usable function of infectivities less than unity in the absence of information about the distribution of numbers of mutants per sample.

The second method differs only in the use of larger cultures of any convenient size, each of which is sampled for the enumeration of mutant phage particles, and a few for the titration of total phage. The mutation rate is given by equation (5), in which h is the average number of mutant phage particles (per culture) giving rise to plaques when C cultures each containing N total phage are plated with an efficiency E on bacteria resistant to the parent phage.

The second method is applicable only if the growth of parent and mutant phage proceeds at identical rates (or, by extension of the theory, if the relative rates are known) during lysis under the conditions of the experiment. One must pay attention especially to the possibility that bacterial mutants may arise in some tubes prior to lysis, on which the mutant phage may grow preferentially, even though there is no selection on the parent bacterial strain. This contingency can be avoided by proper attention to time of sampling. For the rest, it is necessary only in practice to show that in mixed lysates prepared by seeding bacterial cultures with known amounts of both parent and mutant phage, the ratio of the one to the other remains unchanged, except as influenced by further mutations. An example of the use of this method will be shown below

The principal difficulty in applying either of these methods to the measurement of rates of h mutation of phage results from the fact, previously mentioned, that one gets different mutants with different infectivities for the indicator strain used for assay. With *T2H* there are at least two mutants, having infectivities of about 0.2 and 0.8, respectively. These can be differ-

entiated by the appearance of plaques (turbid and clear), but the variation in appearance of plaques of each type prevents a clean separation for counting. It will probably prove possible, by a suitable choice of conditions of assay, to make this separation. In the meantime, we have done preliminary experiments in which we count principally but not exclusively the h^c mutant which serve to answer the question: are *h* and *r* mutations of *T2H* independent of each other.

For this purpose we seed a flask of broth with sensitive bacteria, and with amounts of *T2H* too small to contain any h mutants. The seeded broth is dis-

TABLE 1

Proportion of h mutants arising in stocks of T2H and T2Hr.

PHAGE PER TUBE	6.9×10^9 *T2H*				4.5×10^9 *T2Hr*			
SET NO.	1	2	3	4	1	2	3	4
Tube No.	Clear plaques on *R/2H,2K*				Clear plaques on *R/2H,2K*			
1	3	12	506	131	20	19	88	7
2	9	9	4	3	21	10	35	5
3	3	2	139	227	6	134	5	60
4	10	22	99	4	155	38	16	37
5	5	39	1	260	78	7	41	0
6	189	8	110	31	33	94	1	23
7	8	1	38	68	24	42	18	1
8	0	53	36	516	3	14	1	0
9	23	226	39	113	9	16	0	43
10	237	1	79	14	70	34	86	604
Mean (h)	48.7	37.3	105	137	41.9	40.9	29.1	78.0
Variance (σ^2)	6,910	4,240	19,700	28,400	1,990	1,540	1,020	31,100
σ^2/h, exp.	142	113	187	206	47	38	35	399
σ^2/h, calc.	22.3	18.7	37.8	45.0	20.8	20.0	16.1	30.6
Mutation rate$\times10^9$	1.5	1.2	2.9	3.6	2.1	2.0	1.5	3.4

Each tube contained initially about 10^4 bacteria and about 10^4 phage, in a volume of 0.5 cc After 24 hours, two tubes of each kind were assayed for total phage, and the entire contents of the remaining tubes were plated with resistant bacteria. Only clear plaques were counted (the turbid ones being scarcely visible) and the counts were recorded in random sets of ten. The ratio σ^2/h would be unity if the variations in counts were due to sampling errors alone. The calculated value of this ratio, on the hypothesis of prior mutation, was obtained by the equation (12a) of LURIA and DELBRÜCK (1943).

tributed in tubes to contain 0.5 ml each, and the cultures are then allowed to lyse. Twenty-four hours later the total phage is assayed in a few tubes by plating samples of suitable size on the sensitive bacterium. These counts do not vary appreciably from tube to tube. The entire contents of the remaining tubes are assayed for *h* mutants by plating with the indicator strain *R/2H, 2K* (preferable for this purpose to *R/2H*). To do this one adds to each tube 3.5 ml of 0.6 percent agar containing the bacteria, and pours the mixed contents onto the surface of solidified agar (HERSHEY, KALMANSON, and BRONFENBRENNER

1944). Similar experiments, starting with *T2Hr* instead of wild type, are done in the same way. Further quantitative details, and the results of the assays, are shown in table 1.

The results show that the parallel mutations $T2H \rightarrow T2Hh^c$, and $T2Hr \rightarrow T2Hrh^c$, occur with the same frequency, and therefore that the structural changes in the phage particle corresponding to the *h* and *r* mutational effects occur independently of each other.

It remains only to check whether *T2H* and $T2Hh^c$ compete on an equal basis during growth under the conditions used above. This can be done for both lysis-inhibiting and *r* stocks as follows. Cultures of the sensitive bacterium in broth are seeded with mixtures of host range mutant and corresponding wild type in the proportion of about $1:10^5$. This proportion is sufficiently small to approximate the conditions of the rate measurements, but too large to be appreciably affected by further *h* mutations. The mixture of phages used for seeding is plated on the sensitive bacterium, and on *R/2H*, to enumerate the

TABLE 2

Competitive growth of h^+ and h mutant on E. coli.

	PLAQUES ON *S*	PLAQUES ON *R/2H*	RATIO h^c/h^+
	Mixture of *T2H* and $T2H^c$		
Inoculum	4.8×10^5	4.4×10^0	9.2×10^{-6}
Lysate	3.4×10^{10}	2.8×10^5	8.2×10^{-6}
	Mixture of *T2Hr* and $T2Hrh^c$		
Inoculum	8.8×10^5	2.3×10^0	2.6×10^{-6}
Lysate	1.3×10^{10}	2.8×10^4	2.2×10^{-6}

Plaque counts are expressed per ml of the culture tube.

two component types. After lysis of the seeded cultures, counts are made in the same way of the mixed progeny. The results (table 2) show that no significant change occurs in the proportion of *h* mutant. The two types of phage therefore grow without selective interaction, and the data of table 1 can be used for estimating rates of h mutation.

By equation (5) these rates are, for the mutation $T2H \rightarrow T2Hh^c$, 1.2 to 3.6×10^{-9} in wild type, and 1.5 to 3.4×10^{-9} in *r* mutant, calculated for individual sets of ten cultures, assuming $E = 1.0$. The counts of mutants in individual cultures showed the large fluctuations to be expected for spontaneous mutations in agreement with the experience of LURIA (1945a) with *h* mutations in *T1* and *T2L*.

GROWTH CHARACTERISTICS

One-step growth experiments (DELBRÜCK and LURIA 1942) were made with *T2H* and its *r* mutant. The phages were added to a twofold or greater excess of bacteria, so that the cells were infected with single particles of phage. In one experiment, the simultaneous growth of both phages was followed in a single

tube, counting separately the two kinds of plaque. The experiments, which agreed well, are summarized in table 3. There was no difference in the growth of the two phages except for the delayed lysis in the adsorption tube with *T2H*, and a somewhat larger burst-size with this phage as compared with the *r* mutant. Both phages showed a rather indefinite rise period. The bulk of the phage was liberated during the first ten minutes after the end of the latent period, but this was followed by a gradual rise lasting 20 minutes or more. This indicates sluggish lysis of some of the bacteria, or progressive disintegra-

TABLE 3

Growth of T2H and T2Hr on singly infected E. coli strain H.

PHAGE	NO. OF EXPERIMENTS	ADSORPTION IN 5 MINUTES	LATENT PERIOD	RISE PERIOD	BURST SIZE	LYSIS-TIME IN ADSORPTION TUBE
		percent	minutes	minutes		hours
T2H	2	61	21	30	420	5
T2Hr	3	66	21	30	330	1

tion of bacterial fragments. Evidently the *r* mutation, which alters the rate of lysis of reinfected bacteria in the adsorption tube, does not affect the lysis of singly infected ones in diluted culture.

COMPETITIVE GROWTH AND BACK MUTATION

In contrast with the behavior of the *h* mutants previously described, the *r* mutant shows a decided disadvantage in competitive growth with wild type. Experiments showing this were done with *T2H*. Two tubes containing 10 ml beef extract broth (0.1 percent glucose) were seeded with susceptible bacteria (*H*) and allowed to grow to a population of about 10^7 bacteria per ml. At this point mixtures of the two phages, containing respectively 2 percent *T2Hr* in *T2H* (series A), and one percent *T2H* in *T2Hr* (series B), were added. After lysis was complete, and usually after standing overnight in the incubator, the lysates were freed from any resistant bacteria they might contain by heating at 60°C for ten minutes, samples were plated, and new cultures were inoculated from them to yield a second pair of lysates, and so on. The tubes were seeded each time with about 0.02 ml of the preceding lysate. The two types of phage in mixtures could be counted with fair accuracy by pouring plates with two aliquots yielding about 100 and 500 plaques, respectively. The changes in the composition of the population during several serial transfers with each mixture are shown in table 4. With wild type in excess (series A), the proportion of *r* mutant decreases slowly to the level characteristic of stocks of wild type, near one per thousand. With *T2Hr* in excess (series B), the mutant is rapidly replaced by wild type. The increase in the proportion of wild type under these conditions is twofold or greater for each five-hundredfold increase in phage. This is clearly in excess of the difference in burst-size observed earlier, which could account only for a 30 percent increase.

The competitive advantage of wild type over *r* mutant provides a method for detecting the back mutation. To this end we have made successive serial lysates starting from a single plaque of a pure stock of *T2Hr* (table 4, series C), in parallel with a series propagated under identical conditions starting from a mixture containing only three wild type per 10^8 phage particles (series D). In the latter series, lysis inhibition was evident at the eleventh transfer, and in series C at the twelfth transfer. In both cases, the presence of phage resembling wild type was confirmed by plating and isolating the phage. The line obtained

TABLE 4

Competitive growth of T2H and T2Hr on E. coli strain H.

SERIES	NO. OF SERIAL TRANSFER	LYSIS INHIBITION	PHAGE TITER	PROPORTION OF r^+ PLAQUES
A	inoculum		1.0×10^5	0.98
	1	+	9.3×10^9	0.99
	2	+	4.4×10^{10}	0.99
	4	+	2.7×10^{10}	0.995
	5	+	4.6×10^{10}	0.998
B	inoculum		1.0×10^5	0.01
	1	−	4.6×10^{10}	0.03
	2	−	1.5×10^{10}	0.08
	3	+	2.5×10^{10}	0.1
	4	+	2.6×10^{10}	0.2
	5	+	1.8×10^{10}	0.5
	8	+	1.6×10^{10}	0.96
C	inoculum		fished from plaque	0.0
	1 to 11	−	not plated	
	12	+	6.5×10^9	0.1
D	inoculum		3.0×10^7	3×10^{-8}
	1 to 10	−	not plated	
	11	+	1.4×10^{10}	0.2

in this way proved, on analysis of host range, to be authentic *T2H*. Its identity with wild type was further established by a comparison of rates of mutation to *r* (table 5). Plaques of the back mutant, and of the *r* mutant rederived from it, were indistinguishable from those of the corresponding derivatives of wild type. Back mutation of a similar kind has been observed in every instance tried, including some with genetically marked phages, and the possibility of contamination can be excluded.

The amount of phage transferred from tube to tube in series C and D (table 4) was 0.05 ml, corresponding to about 5×10^8 phage particles. By decreasing this amount until successive transfer would fail to bring out wild type, the rate of back mutation could be estimated roughly—hence this rate must exceed one in 5×10^8. On the other hand, series D reverted faster than series C—hence,

the rate is less than one in 3×10^{7}. The rate of back mutation to wild type is therefore of the order 10^{-7} or 10^{-8}, as compared with about 10^{-3} for the mutation from wild type to *r* (table 5).

An attempt was made to learn something about the competitive interaction of wild type and *r* mutant in individual bacteria by means of experiments similar to those devised by DELBRÜCK (1945a) to demonstrate the mutual ex-

TABLE 5

Proportion of r mutant in lysates of T2H and back-mutant from T2Hr.

LYSATES	TITER	*r* PLAQUES / TOTAL INSPECTED
	$\times10^{10}$	
wild type		
1	1.1	2/640
2	0.89	1/530
3	1.2	0/710
4	1.1	0/640
5	1.4	0/850
Mean	1.1	1/1120
back mutant		
1	1.2	0/730
2	0.96	2/580
3	1.0	0/620
4	0.89	1/530
5	0.63	1/380
Mean	0.95	1/710

Each lysate represents the progeny stemming from a different plaque of the respective phages.

clusion effect. Bacteria (strain H) were allowed to grow in broth to a population of about 3×10^{7} per ml at which time a mixture containing an excess of the two phages was added. After five minutes for adsorption of phage, one sample of the culture was diluted in 0.1 percent peptone water and spun for the assay of unadsorbed phage, and a second sample was diluted 1:50 into 1:100 antiserum (anti-*T2K*). After five minutes for reaction with antibody, during which virtually all the unadsorbed phage was neutralized (DELBRÜCK 1945b), this sample was diluted again by the factor 2500 into broth, and samples were plated with sensitive bacteria both before (aliquots 2 and 5×10^{-6} ml with respect to the adsorption mixture) and after (aliquots 50 times smaller) lysis of the infected bacteria. For comparison, a similar experiment was done with bacteria multiply infected with *r* mutant alone. The results of the three experiments (table 6) were consistent in all important respects; all the bacteria were infected with several particles of phage, and the count of bacteria by colonies or

by infective centers before lysis agreed very well, showing that the free phage had been neutralized. A striking result of mixed infection was the appearance of a new type of plaque, of large size like the *r* plaques, but heavily mottled in characteristic fashion with patches of unlysed bacteria (fig. 1). These proved invariably to contain approximately equal numbers of wild type and *r* phage. Fishings from the typical appearing r^+ or *r* plaques, on the other hand, contained nearly pure wild type, or pure *r* mutant, respectively.

TABLE 6

Mixed infection with T2H and T2Hr (experiments 1 and 2), and multiple infection with T2Hr alone (experiment 3).

DATUM	MINUTES	EXPT. 1 COUNT OF PLAQUES OR COLONIES				EXPT. 2 COUNT OF PLAQUES OR COLONIES				EXPT. 3 COUNT OF PLAQUES OR COLONIES (*r* ONLY)
		r^+	*r*	MIXED	TOTAL	r^+	*r*	MIXED	TOTAL	
Bacteria	−5				57				75	97
Phage added	0	200	360			423	344			1100
Phage adsorbed	5	110	230			312	222			980
Adsorbed per B		1.9	4.0			4.2	3.0			10
Infective centers	<15	3.8	15	40	59	20	15	50	85	91
Phage yield	60	374	620			2780	2480			5800
Yield per B		6.3	11		17	33	29		62	64
r^+/r input		0.56				1.2				
r^+/r adsorbed		0.48				1.4				
r^+/r yield		0.57				1.1				

All counts are expressed per 2×10^{-4} ml of adsorption tube, from the mean of three or four plates each for two different aliquots.

In all three experiments, the yield of phage after one step growth was low, as compared with the yield in single infection. This is probably due to the fact that dilutions were made at room temperature, in 0.1 percent peptone water, and to the poor nutrient conditions during the reaction with antiserum, which was carried out at 37°C in the same fluid. Three additional experiments were done in parallel specifically to compare the yield following multiple infection with wild type alone, *r* mutant alone, and a mixture of the two, under standard physiological conditions. The schedule of the previous experiments was followed precisely, except that certain nonessential measurements were omitted, and the dilutions and reaction with antiserum were all carried out at 37°C in broth. The yield of phage was indeed improved (table 7), though still only about half the yield in single infection. In all other respects the results of these experiments are in excellent agreement with those of table 6.

The conclusions to be drawn from the experiments with multiple infection may be summarized as follows.

As one varies the proportionate input of *r* mutant to wild type, the number of mixedly infected bacteria yielding wild type alone, or *r* mutant alone, varies

TABLE 7

Simultaneously Measured Yields of T2H and T2Hr in Multiple Infection.

INFECTING VIRUS	*T2Hr*	*T2Hr* AND *T2H*			*T2H*	
PLAQUE-TYPE	*r*	*r*+	*r*	MIXED	*r*+	*r*
Bacterial count	109					
Phage added*	700	350	350		700	(1)
Adsorbed per B*	4.8	2.4	2.4		4.8	
Infective centers at 12–20 Min.	127	17	25	75	(317)†	(1)
Yield at 60 Min.:						
Growth tube 1	16,300	9,800	10,300		16,600	(200)
Growth tube 2	15,200	8,300	9,100		18,800	(100)
Yield per B	130	75	80		147	(1)
r+/*r* input		1.0				
r+/*r* yield		0.94				

* Input adjusted from prior counts: 75 percent adsorption assumed

† Sample plated at 21–22 minutes; evidently growth of phage had begun. See legend table 6.

in the expected direction. These numbers are about equal for 1:1 infection, indicating no superiority of the one over the other with respect to mutual exclusion (DELBRÜCK 1945a). This is evidently another expression of the near relatedness of the two phages.

The mutual exclusion effect itself is weak, for the majority of bacteria yield both types of phage, and evidently both grow. Another interpretation might be made in terms of directed mutation, under the influence of excluded virus, among the progeny of a single successful particle (DELBRÜCK 1946b). The quantitative data, particularly the identity of input and yield ratios following mixed infection, do not support this interpretation, but do not really decide the question.

The ratio of wild type to *r* mutant resulting from one-step growth in mixedly infected bacteria is identically equal to their ratio in the infecting mixture for the three ratios tested—namely, 0.6, 1.0, and 1.2. From the other data, and since the mixed yielders comprise 59 to 68 percent of the mixedly infected bacteria, the mixed yielders as a class must also conform to this rule.

The total yield of phage from bacteria multiply infected with wild type alone, *r* mutant alone, or a mixture of the two, is the same; about 150 particles per bacterium. There is, therefore, no depressor effect (DELBRÜCK 1945a), and the two phages grow as if they were competing on an equal basis for a common substrate, without other interaction.

The total yield of phage in multiple infection is about half that found in single infection, with either wild type or *r* mutant. However, the two sets of experiments were separated by an interval of about a year, and we have not determined whether the difference is an effect of multiple infection itself, of the antiserum, or of some unknown variable.*

The experiments on mixed infection under defined conditions do not reveal any mechanism adequate to explain the superiority of wild type over *r* mutant during competitive growth in mixed culture. The tendency for pure stocks of wild type to reach higher titers in broth, noticeable in tables 1 and 2 and consistently seen with other even-numbered phages also, is probably related to the selective effect in mixed culture, but it likewise is without explanation.

HOST SPECIFICITY

The question of the identity of host range of wild type and *r* mutant was investigated very thoroughly for all the even-numbered phages of the *T* system and for several *h* mutants of *T2H*, comprising eight r^+ stocks in all, and their corresponding *r* mutants. With each of these, ten or more independently arising phage-resistant bacterial mutants were isolated, by methods similar to those of DEMEREC and FANO (1945). As noted by these workers, mutants of *B* resistant to *T2* are difficult to obtain. These were isolated with little difficulty, however, by first growing sensitive bacteria in the presence of phage in salt-free broth for a few days (which selects partially resistant mutants), and then transferring phage and bacteria to broth containing 0.5 percent salt. After incubating these cultures for about two days, streak plates from them nearly always yielded stable resistant mutants.

The principal types obtained have already been mentioned in the section of this paper headed "materials." Other types included these same patterns in combinations of two or more linkage groups. Among these the most frequent were R/*2, 3, 4;* R/*2, 3, 4, 7, 16;* R/*2, 3, 4, 6, 7, 16;* and R/*2, 6, 16.* As in the other cases, resistance to *T2* nearly always included *T2H*, *T2K* and *T2L*.

Tests of sensitivity to phage were made by flooding the surface of agar plates with broth cultures of the bacteria to be tested, drying the plates by inverting them in the incubator with covers removed for one hour, and then placing small drops of suitably diluted phage from capillary pipettes on the surface of the seeded agar. The drops were evaporated down by again exposing the plates for 20 minutes in the incubator. In many cases several dilutions of the phages were tested. A culture was arbitrarily classified as resistant when a drop of phage containing about 10^6 phage particles produced no marked confluent lysis and yielded fewer than 500 plaques. Usually there was no effect whatever at this dilution on resistant cultures, and more or less complete lysis with sensitive ones.

* Subsequent experiments have shown that certain lots of nutrient broth give low yields of virus, the maximum for *T2H* being about 400 particles per bacterium in single infection. Using a given lot of broth, the yield is reproducible, is not affected by antiserum, and is larger rather than smaller following multiple as compared with single infection.

None of the 200 or more cultures tested, including some dysentery strains, and some derivatives of strains of *E. coli* unrelated to *R*, distinguished sharply between any wild type phage and its *r* mutant. With some incompletely resistant strains, there were quantitative differences in infectivity of the two types. This kind of difference is of doubtful significance for the reason that similar differences are seen between different stocks of the same type, depending for one thing on the proportion of *h* mutants they contain.

One extreme case of this kind was examined in detail. The bacterial strains *R/2K, 6, 16* and *R/2H, 2K*, resistant to *T2K*, proved to be sensitive to our stock of *T2Kr*. But when new stocks of each type were prepared, starting from a single variegated plaque of wild type, the difference disappeared. We conclude that our stock of putative *r* mutant had also undergone an *h* mutation, either before or after its origin from the r^+ type. The results of tests with these stocks are shown in table *8*, which will serve to illustrate the method we have used for analysis of host specificity. Tests with *T2H* and *T2L* are included for comparison.

TABLE 8

Spot tests on seeded agar with T2K and T2Kr.

BACTERIAL STRAIN		*R*	*R*	*R*	*R*	*D*
PHAGE	TITER PER DROP	*2H, 2L*	*2H*	*2H, 2K*	*2K, 6, 16*	*2K, 3, 4, 7*
T2K	3×10^8	C	C	—	2	—
T2Kr1	3×10^8	C	C	C	C	1
T2Kr2	3×10^8	C	C	—	1	—
T2K	3×10^4	>1000	100	—	—	—
T2Kr1	3×10^4	>1000	1000	200	>1000	—
T2Kr2	3×10^4	>1000	50	—	—	—
T2K	3×10^2	30	—	—	—	—
T2Kr1	3×10^2	100	20	2	100	—
T2Kr2	3×10^2	20	—	—	—	—
T2H, r^+ or *r*	3×10^5	—	—	—	C	C
T2L, r^+ or *r*	3×10^5	—	C	C	C	C

T2Kr1 was isolated in 1944; *T2Kr2* is an independent mutation recently isolated from *T2K*. *D/2K, 3, 4, 7* is a wild type *Shigella paradysenteriae*.

The symbol C indicates complete lysis, numbers refer to plaques, and — indicates no effect.

Since the tests described above were completed, we have had occasion to examine a rather large number of independently arising *r* mutants of several phages. These have never differed in host range from the parent stock, which makes it unlikely that coupled *h* and *r* mutations ever occur.

In general, the analysis of host specificity of r^+ and *r* phages has merely confirmed our earlier conclusion that the *h* and *r* genetic factors of bacteriophage are mutually independent.

ANTIGENIC SPECIFICITY

Neutralization tests with several antisera failed to show any antigenic difference between wild type and *r* mutant. Absorption tests were therefore resorted to, the results of which will be given here only in condensed form.

The methods of serological analysis used in these experiments have been described in detail elsewhere (HERSHEY, KALMANSON, and BRONFENBRENNER 1943). They are based on the measurement of the specific rate of neutralization (k) per second, which for the present purpose may be defined as a number proportional to (1/2240 ×) the dilution of serum which will neutralize 80 percent of added phage in one hour under specified conditions. This measurement can easily be duplicated with an accuracy of 20 percent. With homologous phage and antiserum, k provides a measure of the antibody content of the serum. In heterologous reactions, the measured k is a plausible index of antigenic relatedness.

It was shown by BURNET (1933b) that absorption with heterologous phage increases the specificity of the antiserum, as is the case with other antigens. He used phage adsorbed to killed bacteria for the absorbing antigen. His method is of limited applicability for the reason that many phages, *T1* for instance, are not adsorbed to dead bacteria. We have made adsorptions with phage alone, which is practicable at least with *T1* and the even-number phages of the *T* system.

For the even-numbered phages, the appropriate conditions for absorbing 50 to 80 percent of the antibody from antisera at 37°C in broth containing 0.5 percent NaCl are met when the ratio of antibody to phage is

$$\frac{\text{ml antiserum} \times \text{k}}{\text{lytic units of phage}} = 2 \times 10^{-11}$$

The mixture should contain about 10^{10} lytic units per ml, in which case precipitation occurs in a day or two, and the sediment can be removed before titration. With more dilute mixtures, no visible precipitate forms, but the results of titration are very similar. At low temperatures the reaction is slower, but considerably more antibody is absorbed at equilibrium. The specified conditions are equally suitable for homologous or heterologous absorption, provided the appropriate k is substituted in the formula given above. Mixtures prepared under these conditions contain only negligible amounts of active phage, but if it is desired to avoid this, one can carry out the absorption with phage previously inactivated with minimal amounts of formaldehyde.

The results of tests made with several phages are summarized in table 9. The data are expressed in terms of the ratio of the specified heterologous k to the homologous. For example, *T4* in whole anti-*T2K* yields k = 1.0, and *T2K* in the same serum yields k = 4.5, the ratio 0.22 being entered in the table. This ratio expresses the relatedness of the phages on the one hand, and on the other measures a quality of the antibody, a lower ratio indicating greater specificity. It may therefore be taken as a criterion of antigenic difference between phages,

that heterologous absorption will lower this ratio of k's. This effect is more significant than the absolute value of the ratio, which might conceivably be influenced by causes other than antigenic dissimilarity (no case of this kind has been encountered, however).

Similar considerations apply to the reliability of measurements. For instance, the ratio 1.3 recorded in table 9 for the neutralization of *T2H* by anti-*T2K* is certainly not significantly different from unity, since it is the consequence of a single aberrent result (1.6) among three titrations and is in any case a deviation in the opposite direction to that found with heterologous phages. Accordingly, one finds no significant effect on this ratio of absorption with *T2H* or *T2L*, all three titrations being influenced by the same error. This is an expres-

TABLE 9

Antigenic relationships among even-numbered and mutant phages.

SERUM-FRACTION TESTED	RATIO OF HETEROLOGOUS TO HOMOLOGOUS TITER AGAINST					
	T2H	*T2Hrk*^{c}	*T2L*	*T2Kr*	*T4*	*T6*
Anti-*T2K*, whole	1.3$_3$	0.97$_1$	0.72$_3$	0.96$_7$	0.22$_3$	0.20$_2$
Anti-*T2K*/*T2H*	1.3$_2$					
Anti *T2K*/*T2L*	1.3$_1$		0.60$_3$		0.40$_3$	
Anti *T2K*/*T2K*				0.95$_3$	0.67$_2$	
Anti-*T2K*/*T2Kr*				0.93$_7$	0.69$_2$	
Anti-*T2K*/*T4*	1.0$_1$		0.87$_6$	1.1$_2$	0.04$_3$	0.03$_2$
Anti-*T2K*/*T2Hrk*^{c}		1.2$_4$				
Anti-*T2Hr*, whole	1.03$_2$					
Anti-*T2Hr*/*T2H*	0.94$_2$					

Anti-*T2K*/*T2H* means the serum of a rabbit immunized with *T2K* from which 50 to 90 per cent of the antibody has been absorbed with *T2H*, etc.

The subscript numerals indicate the number of independent measurements of the specified ratio contributing to the average result shown.

sion of the technically important fact that simultaneous titrations of two antisera with the same phage are subject to less relative error than simultaneous titrations of two phages in one antiserum, or successive titrations of the same materials (HERSHEY, KALMANSON, and BRONFENBRENNER 1943). For this reason, we arrange our titrations to include in each run the sera it is desired to compare, against the homologous and one or two heterologous phages.

From the same considerations (and since a similar relationship was evident in each of the individual titrations), the very small effects of heterologous absorption on the neutralization of *T2L* can be taken as evidence of a slight antigenic difference between this phage and *T2K*.

Summarizing the data of table 9 we find:

T2K and *T2H* are indistinguishable in anti-*T2K* antiserum. *T2L* is slightly, and *T4* and *T6* are very much, different from these. *T16* (data not shown) is still further removed, judging by rate of neutralization alone.

T2Hr and *T2Kr* are indistinguishable from the corresponding wild types in antiserum against either mutant or wild type.

An *r* mutation followed by *h* (*T2Hrh*^{c}) does not result in any cumulative antigenic difference. This conforms with our failure to distinguish *h* mutant from wild type *T1* by absorption test (data not shown).

Absorption of anti-*T2K* with *T4* removes selectively antibody which reacts with *T4* and *T6*, but not (or less rapidly) with *T2L*. This implies a component antigenic pattern common to *T2K*, *T4*, and *T6*, which is different in *T2L*.

Absorption of anti-*T2K* with *T2L* removes selectively antibody which reacts with *T2L*, but not with *T4*. This implies an antigenic component common to *T2K* and *T2L*, which is different in *T4*.

Absorption of anti-*T2K* with *T2K* (or *T2Kr*) leaves behind a fraction of antibody that reacts almost equally well with *T4* and *T2K*. This confirms in a remarkable way some of the conclusions stated above.

T4 and *T6* are indistinguishable in anti-*T2K* antiserum, although they are easily distinguishable in their own. Evidently these two phages have the same antigenic component in common with *T2K*. This component is probably not associated with the *r* locus, for *T4* and *T4r* cannot be distinguished in anti-*T2K* after absorption with either one (data not shown). The relationship of *T16* with respect to this component could not be conveniently determined, because of the very low titer of anti-*T2K* against this phage.

DISCUSSION AND SUMMARY

Phages belonging to the *T2* (even-numbered) series, form a group that is morphologically homogeneous and whose members are antigenically related; they are dependent for their infectivity on relatively high concentrations of univalent cations, which in general inhibit other members of the *T* system (unpublished experiments), and they are characteristically sensitive to urea, but are comparatively resistant to heat and certain other agencies (BURNET 1933c).

In addition, these phages as encountered in nature exhibit a unique lysis-inhibiting property recognizable by delayed lysis in broth and only partial lysis in the peripheral zone of high phage concentration surrounding plaques in agar. From the wild types (*r*$^+$) one regularly obtains mutants (*r*) which fail to show either of these lysis-inhibiting effects, or show them to much smaller degree. The frequency of the *r* mutation is about 10^{-3} or 10^{-4}.

The *r* mutant, in turn, mutates back to wild type, but with much lower frequency, probably about once per 10^8 duplications. The important question, whether this is a true back mutation—that is, whether the recovered lysis-inhibitor is identical with the original wild type—seems to be answered in the affirmative by the finding that both mutate to *r* with similar frequency. This latter evidence is reasonably decisive and leads to the conclusion that *r* mutation corresponds to a true allelic modification of structure rather than to loss of any genetic material.

When mixtures of wild type and *r* mutant are propagated in broth on bacterial strains equally sensitive to the two types by all other tests, wild type replaces *r*. Although the mechanism of this replacement is unknown, it is

evident that it explains the persistence of wild type in spite of the unfavorable balance of mutation rates. It also permits the demonstration of the back mutation, which would otherwise not be observable.

A mutation resulting in altered host range ($h^+ \rightarrow h$) has been found to occur at the same rate in r and r^+ stocks of one of these phages (*T2H*). This may be taken as proof of the existence of at least two independent genetic factors, and it is permissible to refer to these as h and r loci, provided the method of identification is kept in mind.

The existence of independent genetic factors does not alter the status of the analogy commonly drawn between viruses and genes. The mutational properties of the phage are precisely analogous to those of the *R* determinants of seed and plant color in maize, which always segregate together, but undergo mutation independently (STADLER and FOGEL 1943). In either case one has freedom of choice in speaking of multiple genes (that is, mutational units), or of independent functions of a single gene (the crossover unit). Evidently, with viruses as with bacteria (DEMEREC and FANO 1945), the only genetic factors amenable to study by present methods are the mutational units. For the present, then, no useful distinction can be made between the genetic behavior of a bacterium, a virus, and a crossover unit bearing two or more independently mutating genetic factors.

The antigenic specificity and the host specificity of wild type and r mutant are the same, and h mutation alone, or successive to r, also fails to alter serologic character. Evidently these three hereditary characters of the virus are independent of each other, or nearly so. The independence of h mutation and antigenic specificity is in keeping with what might be surmised from numerous instances of adaptation to unnatural hosts among the animal viruses, where a similar principle forms the basis of the classical methods of antiviral prophylaxis.

In connection with the serological experiments, an essay was made toward antigenic analysis of relationships among natural phage types by means of cross absorption tests. The results show that the methods available would permit the serological structure of bacterial viruses to be dissected in great detail. The conclusions from the preliminary experiments have been summarized in the experimental part of this paper and will not be repeated here.

Experiments in which bacteria are simultaneously infected with wild type and r mutant show the unexpected result that many bacteria yield both types of phage. For instance, if each bacterium receives about three particles of each type of phage, the population splits into about 20 percent wild type yielders, 20 percent r yielders, and 60 percent mixed yielders. The mixed yielders produce wild type and r mutant in the same ratio with which the bacteria were infected and in an amount which suggests that the two types are initiating growth and competing with equal efficiency for the same substrate. The proper interpretation of these results is not yet clear, however.

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SUMMARIES FROM FORTHCOMING PAPERS

GORDON, MYRON. *Genetics of Platypoecilus maculatus. Sex determining mechanism in two wild populations of the Mexican platyfish.*—Received June 25, 1946.—The sex-determining mechanism in two wild populations of the Mexican platyfish, *Platypoecilus maculatus*, one from the Rio Papaloapan and the other from the Rio Jamapa, both in the state of Vera Cruz, may be expressed as follows: XX = female, XY = male.

The domesticated stocks of the same species have a sex-determining mechanism which may be expressed as follows: WZ = female, ZZ = male. It is suggested, in view of the probable origin of this mechanism from the wild type that the formula might better be written: WY = female, YY = male.

An instance of sexual transformation of a genetically constituted female of the "wild" stock XX has been reported. When this XX male was mated with a normal "wild" type female, also XX, all the offspring, 153 in all, were females, XX.

SCHMIEDER, R. G., and P. W. WHITING. *Reproductive economy in the chalcidoid wasp Melittobia.*—Received July 17, 1946.—In an undescribed species of the chalcidoid genus Melittobia close inbreeding is the rule, but outcrossing may occur. Unmated females lay very few eggs, and these are male-producing. After mating occurs, an abundance of eggs are laid which, for the most part, develop into females, males constituting less than three percent of the offspring.

The experiments including egg counts show that despite close inbreeding with "selfcrosses" of haploid sons back to mothers, there are no diploid males and no "bad" eggs as in Habrobracon sex homozygotes. Moreover, outcrosses have been less fecund than closecrosses. The methods of reproductive economy and of sex determination have not been determined.

Fraternities fall into two groups as regards successive broods on different hosts—the uniform with broods of high fecundity and the fluctuating with some broods of very low fecundity. These differences appear not to be caused by food but may be genetic.

It is suggested that in haplodiploid species multiple sex-allelism may be the more primitive and general method of reproductive economy and that the closecrossed species have adopted some other method.

KODANI, MASUO. *Variations in the terminal bands of the salivary X chromosome of Drosophila melanogaster.*—Received July 1, 1946.—A few terminal bands in the X chromosome of the salivary gland nucleus appear to be absent in some mutant and wild stocks of *Drosophila melanogaster*. These bands are actually present in the chromosome, but are closely apposed to the more proximal band and do not appear as separate individual bands. Some or all of these bands appear as independent bands when the chromosome tip is paired. A chromosome tip may influence the tip of its pairing partner so that the hidden bands may appear. The chromosome tips of some stocks exert a greater influence of this sort on the tips of the partner chromosome tips than those of others. A possible mechanism for the separation of the compound bands into individual bands is suggested.

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BEARING ON HEREDITY AND VARIATION

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Page 209, line 33, for "Kirilov" read "Kirikov."
Page 213, line 8, for "map of the Ukraine" read "map of a part of the Ukraine."
Page 214, line 8, for "near Odessa" read "near the Crimea."
Page 225, table 7, top of right column, for "1.97" read "1.07."
Page 229, line 40, for "figures 1 and 2" read "figures 4 and 5."
Page 247, line 16, for "of table 6" read "of table 5."
Page 250, line 14, for "is nearly" read "is more than."
Page 567, line 20, for ".00007" read ".001."

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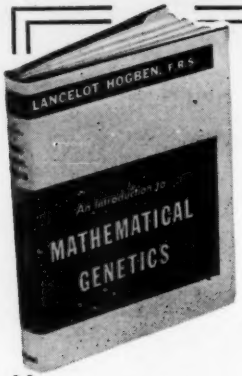

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